EDGEWOOD ARSENAL
TECHNICAL REPORT

EATR 4108

THE TOXICOLOGY OF DM

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W. U. Thomas, T. L. Hess, R. L. Farrand, S. G. Ryan
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October 1967

DEPARTMENT OF THE ARMY
EDGEWOOD ARSENAL
Research Laboratories
Medical Research Laboratory
Edgewood Arsenal, Maryland 21010

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FOREWORD

The work described in this report was authorized under Project 1C522301A079, Non-Defense Medical Aspects of Chemical Agents (U). The work was started in April 1965 and completed in September 1966.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

Acknowledgments

The authors acknowledge the assistance of personnel of the Basic Toxicology Branch and the Biostatistical Section for the statistical evaluations and the assistance of Mrs. Martha Langan in the preparation of the charts, graphs, and tables contained herein and for the large amount of secretarial work.

DIGEST

This report summarizes the toxicological testing of diphenylaminochloroarsine (DM) in animals during the period from 1918 to 1965. Included are determinations of the toxicity of the compound disseminated by laboratory methods in early work and from military and commercially available thermal munitions in later work. The most probable human LCt50 estimates are derived from these experiments for the various methods of dissemination. All work described under the animal testing section of the report pertains to either field or chamber whole-body exposures of eight species of test animals. Other portions of the toxicity studies deal with the pathological changes in exposed animals, times to death, and toxic responses.

All available information on human exposure to DM, including accidental exposure of US and alien troops and Army personnel, is included.

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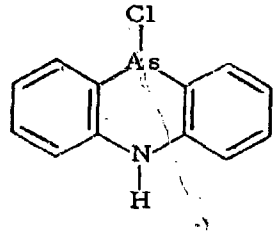
THE TOXICOLOGY OF DM

I. INTRODUCTION.

DM is the code name for diphenylaminochloroarsine. It is also known as G-322, chlorodihydrophenarsazine, arsenic sneeze, and adamsite. It was synthetized by an American, MAJ Roger Adams, in 1918. It is one of a series of compounds known as toxic smokes, irritant smokes, sternutators, or sneeze gases by the US, and as Blue-Cross gases by the Germans. The chemical formula is:



The compound is a canary-yellow crystalline solid when pure, but dark green when impure. It melts with slight decomposition at 195°C and boils at 410°C at 760 mm Hg. It is insoluble in water and moderately soluble in organic solvents.[1-5]

DM can be dispersed as an inhalable aerosol from pyrotechnic mixtures and from solvent sprays by volatilization and condensation. It can also be dispersed as a preground dry powder.

The initial biological data on DM were developed during and immediately following World War I. At that time, the irritating and incapacitating effects in man were studied by Lawson and Temple,[6] Eldridge,[7] and others.[4, 8] Human and animal data developed prior to 1922 were reviewed by Craighill and Folkoff.[9]

Between 1922 and 1957, little work was done with the compound. In 1957, Wilding and coworkers* conducted a series of human exposures to determine the tolerable inhalation dose. In 1958, Gongwer and coworkers[10] compared the effectiveness of chloroacetophenone (CN), pelargonic morpholide, and DM in human subjects.

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* Wilding, J. L., et al. Aerosol Branch. 1957. Unpublished data.

DM inhalation experiments were conducted between 1957 and 1964 with rodents, dogs, and monkeys to determine the relative toxicity of a particular sample of agent that was intended for filling chemical munitions. The results of these studies were highly variable.

In April 1965, the Presidential Scientific Advisory Committee, following a review of the available toxicity information on DM, requested that a definitive series of toxicity studies be performed to characterize the agent when it was dispersed by laboratory methods or from standard and commercial thermal munitions. Between April and September 1965, these investigations were performed by the Aerosol Branch, Toxicology Department.

II. EFFECTS IN MAN.

A. Incapacitation.

The onset of signs produced by exposure to DM aerosols may be immediate or may be delayed for several minutes. The initial effect is irritation, followed by a burning sensation and pain in the eyes, nose, throat, and respiratory tract. Uncontrolled coughing and violent, persistent sneezing occur. Lacrimation and copious flow of saliva are produced. Congestion appears in the conjunctiva, nose, and pharyngeal wall. These signs of irritation subside, and after 20 or 30 min, headache, mental depression, perspiration, chills, nausea, abdominal cramps, vomiting, and diarrhea may appear. Most of these effects disappear within a few hours.[1, 8-13]

DM has been used effectively as a method of quelling riots, and thousands of humans have been exposed to it. However, the few human deaths that have occurred indicate that there is the risk of a few sensitive individuals dying, especially if the agent is used in inclosed areas fron which escape is not possible.

B. Minimal Effective Dosages.

The lowest concentrations (sprayed from alcoholic solutions) that are irritating to the throat and lower respiratory tract are 0.38 and 0.5 mg/cu m, respectively. The lowest concentration causing cough is 0.75 mg/cu m.[7]

C. Effective Incapacitating Dosages.

1. Laboratory Tests.

Data sheets (from the files of the War Department, Chemical Warfare Service, Edgewood Arsenal) relating to work with DM prior to March 1921 were reviewed by Craighill and Folkhoff.[9] The data in table I were taken from this source or from the original reports.

Lawson and Temple[6] developed a curve for tolerance time* at various concentrations of DM. Several points on the curve for intolerable concentrations for man are listed below. Additional results of this study are shown in table II. (The data of Lawson and Temple may also be found in the review by Craighill and Folkhoff.[9])

Intolerable concn mg/cu m	Time min
49.00	0.75
22.30	1
5.80	2
2.20	3
1.00	4
0.72	5
0.30	10
0.23	15
0.19	20
0.17	30
0.14	60

In these tests, the agent was administered through a mask and not by whole-body or whole-head exposures of the volunteers. The following symptoms were reported to Lawson and Temple[6] by the subjects used in gathering the data listed in table II. Their descriptions are verbatim.

> Immediate Effects - The earlier symptoms were relatively light. Burning and irritation of nose and throat were first felt. This was often accompanied by a slight irritation of eyes and lachrymation. The affected area seemed gradually to spread downward into the chest, causing a warm and

* Time at which the subject could no longer tolerate exposure and left the chamber.

Table I. DM Human Tolerance Tests—Closed Chamber Trials

Test No.	Pulse		Respiration		Time symptom occurred		Time of tolerance*	Subsequent symptoms	Approx intolerable Ct**
	Before	After	Before	After	Irritation	Cough			
	bpm		breaths/min		min, sec				
A. Concentration, 2 mg/cu m (0.002 mg/l)									
1	92	92	16	16	2, 15	5, 50	5, 50	Nausea, coughing, salivation	12.0
2	68	72	16	16	1, 45	None	3, 0		6.0
3	64	64	16	16	0, 35	2, 20	2, 20		4.6
4	88	100	20	20	0, 50	2, 50	6, 20		12.6
5	112	120	16	16	1, 0	1, 30	2, 50		8.0
6	92	92	16	20	1, 45	2, 35	5, 32		11.0
7	72	100	20	20	0, 45	1, 0	2, 40		4.6
8	92	100	16	20	0, 30	3, 25	15, 0		30.0
9	96	104	16	24	1, 0	4, 30	7, 50		16.0
10	84	104	16	24	3, 0	None	6, 40		15.6
11	92	100	20	20	1, 30	1, 30	3, 15		6.5
12	68	76	16	16	1, 0	3, 30	4, 40		9.2
13	72	96	16	20	2, 50	None	15, 0		30.0
14	96	100	16	20	1, 0	5, 0	5, 0	Burning throat, perspiration for 1 hr	10.0
15	120	120	16	16	1, 30	None	8, 0		18.0
16	104	112	16	20	2, 50	2, 50	3, 10	Nausea, salivation, mental depression	6.0
17	76	76	16	16	1, 15	3, 40	3, 40	Nausea, dizziness, headache	7.2
18	64	76	16	16	0, 30	3, 45	9, 0	Coughing, burning, headache	18.0
19	92	92	16	16	0, 30	3, 30	3, 20	Headache, sneezing, perspiration	7.0
20	64	70	20	28	2, 10	None	7, 30		15.0
21	84	96	20	28	0, 30	None	15, 0		30.0
B. Concentration, 5 mg/cu m (0.005 mg/l)									
1	80	80	16	16	0, 50	None	1, 20	Slight perspiration, nasal discharge, burning throat	6.5
2	84	84	20	20	1, 15	4, 0	5, 0	Perspiration, burning; recovery in 1-1/2 hr	7.5
3	94	80	16	16	1, 15	4, 0	5, 0		25.0
4	96	96	20	20	2, 30	3, 30	5, 30	Sneezing, coughing, tightness in chest; recovery in 2 hr	27.5
5	80	80	20	20	0, 30	0, 50	1, 15	Sneezing, coughing, tightness in chest; recovery in 1 hr	6.25
6	88	88	16	16	1, 20	1, 0	2, 45	Nausea; recovery in 4 hr	13.75
7	64	64	20	20	2, 30	4, 20	4, 20	Weakness, coughing, burning	21.50
8	76	112	16	28	1, 40	2, 20	12, 30	Nose bleeding, headache persisting 13 hr	62.5
9	76	80	16	20	1, 0	2, 30	5, 0	Nausea	25.0
10	60	96	16	20	1, 45	2, 10	2, 45	Sneezing, coughing, tightness in chest	13.75
11	84	100	16	20	1, 0	2, 30	4, 0	Sneezing, coughing, tightness in chest	20.0
12	80	80	16	16	1, 0	1, 30	2, 0	Sneezing, coughing, tightness in chest	10.0
13	72	76	16	16	0, 30	None	0, 45	Sneezing, coughing, tightness in chest	3.75
14	76	84	20	20	0, 20	0, 20	1, 30	Sneezing, coughing, tightness in chest	7.50
15	80	84	16	16	1, 0	2, 0	2, 0	Sneezing, coughing, tightness in chest	10.0
16	80	84	16	20	2, 30	5, 0	5, 0	Sneezing, coughing, tightness in chest	25.0
17	112	132	20	24	0, 20	None	1, 10	Sneezing, coughing, tightness in chest	5.0
18	64	68	16	16	2, 10	5, 0	5, 0	Sneezing, coughing, tightness in chest	25.0
19	68	100	16	16	0, 50	1, 40	2, 10	Sneezing, coughing, tightness in chest	10.0
20	92	104	20	20	1, 10	None	2, 0	Sneezing, coughing, tightness in chest	10.0
21	96	120	16	20	1, 20	2, 0	2, 10	Sneezing, coughing, tightness in chest	10.0
22	72	72	20	20	0, 15	2, 30	2, 30	Sneezing, coughing, tightness in chest	12.5

Note: The concentrations were inaccurate because of precipitation in the chamber. One-half the original amount of DM was added every 5 min to replace this loss. Part of the substance was decomposed by heating it on the hotplate. The method was rough, but it gave the general trend of the tolerance time.

* Time at which subject could no longer tolerate agent and left the chamber.

** Calculated in Toxicology Division, Edgewood Arsenal, 1965.

Table II. Relation Between Concentration and Limit of Tolerance of Man for DM

Concn	No. of men exposed	Av limit of tolerance	Ct	Limit of tolerance for individuals
mg/cm m		sec	mg min/cu m	sec
61	6	41	41	50, 40, 40, 45, 50, 20
45	7	46	35	35, 40, 35, 50, 40, 60, 60
22	6	56	20	45, 55, 55, 75, 70, 35
14	10	77	18	60, 45, 30, 120, 45, 80, 80 105, 105, 105
6	6	123	12	180, 140, 165, 105, 90, 60
2	5	168	6	270, 135, 135, 120, 180
1	7	235	4	210, 280, 255, 240, 180, 330, 150
0.9	5	235	4	135, 225, 255, 270, 290
0.5	5	390	3	660, 360, 210, 450, 270
0.2	4	668	2	360, 616, 570, 1,125
0.2	5	672	2	600, 870, 675, 780, 435
0.2	4	1,552	5	3,090, 600, 1,020, 1,500
0.1	1	3,600	6	3,600
0.1	2	3,600	6	3,600, 3,600

tingling sensation and eventually a short rasping, annoying cough. This was almost coincident with the first feelings of distress. It was at this point that the mask was usually removed. With low concentrations where the exposure was for several minutes or more, nasal irritation was generally followed by nasal discharge.

Aftereffects - The more severe symptoms were usually felt after exposure, when the subjects began to breathe fresh air. In most cases with extremely low concentrations the after effects were scarcely noticeable. As the concentration was increased, the after effects increased, varying, however, rather in degree than in nature. In the case of high concentration, they were particularly severe, causing acute distress. There was tightening and burning across the chest accompanied by a feeling of suffocation, and a persistent short rasping cough and acute general depression. These effects reached a maximum in about 10 minutes, after which there was gradual relief. The period of distress varied from zero with the lowest concentration to from 2 to 3 hours with the highest concentrations.

Delayed Effects - Delayed effects were infrequent, an occasional dull headache persisting for several hours, and in one case, where the concentration was 0.06 mgm/liter a man was incapacitated for work for 2 days, with stomach trouble, dull headache, and general depression. A few other cases were found where stomach trouble was caused by the gas, du due, in the writer's opinion, to individual susceptibility.

2. Field Tests.

Data from several field tests, conducted before 1922, are contained in the review by Craighill and Folkoff.[9]

In the first DM cloud test, DM cloud generators were placed on an 85-yd front to give a DM distribution of 2 lb/yd of front. Sixteen canisters containing a total of 11 lb of DM failed to ignite, and considerable DM was deposited on the ground from the cloud along the entire front. The time of evolution was 25 min. Samples of air were taken at various distances from

the generators and analyzed. The averages of four samples taken across the center of the line of discharge at the time of maximum cloud density are:

Distance from source, yd	Concn, mg/cu m
500	12.29
1,000	4.95
1,500	3.54
2,000	2.47

Forty guinea pigs were placed at various distances in the path of the cloud at 7-yd intervals across it. Men were stationed 1,000, 1,500, 2,000, and 2,500 yd from the source. The results of these tests are given in tables III and IV.[9]

Table III. Effects of DM on Guinea Pigs in First Field Test

Distance from source, yd	No. of guinea pigs	No. killed	No. affected*	No. not affected
50	10	1	4	5
100	10	—	7	3
200	10	—	3	7
500	10	—	1	9
Total	40	1	15	24

* Guinea pigs that showed irritant effects.

In the second test, 53 lb of DM (dispersed from candles) were used on a 200-yd front. The time of evolution was 10 min, the wind velocity was from 5 to 10 mph, and the temperature was 58°F. The results of this test are given in table V.[9]

In the third test, 600 canisters (each containing 10 oz of DM) in 55 groups of 12, were distributed over a 60-yd front. The time of discharge of each canister was 2-1/2 min; one canister in each group was ignited every 2-1/2 min. Excluding duds, 166 lb of DM were fired. The results of this test are shown in table VI.[9]

Table IV. Effects of DM on Unprotected Subjects in First Field Test

Distance from source	Time first detected	Effects on observers	Remarks	Approx exposure Ct*
yd	min			mg min/cu m
1,000	—	Severe symptoms during and after exposure	Retired in 20 min	100
1,500	4.5	Moderate symptoms	Did not retire	16
2,000	7	Marked symptoms before end of experiment		17
2,500	8 - 9	Moderate symptoms	Did not retire	—

* Calculated in Toxicology Division, Edgewood Arsenal, 1965.

Table V. Effects of DM on Unprotected Subjects in Second Field Test

Distance from source	Concn	Effects on observers	Approx exposure Ct*
yd	mg/cu m		mg min/cu m
500	24.78	Symptoms produced; retired in 6 min	149
1,000	12.39	Light symptoms	124
1,500	8.26	Very light symptoms	83

* Calculated in Toxicology Division, Edgewood Arsenal, 1965.

Table VI. Effects of DM on Unprotected Subjects in Third Field Test

Expt No.	Amount of DM fired	Time of evolution	Distance from source	Concn	Effect on observers	Approx exposure Ct*
	lb	min	yd	mg/cu m		mg min/cu m
1	6-1/2	10	200	7.08	Severe symptoms in 5 min	35
			400	–	Incapacitated for over 15 min	
			1,200	–	Light symptoms	
2	3-3/4	9	200	9.91	Pronounced effects; retired in 5 min	50
			1,200	–	Light symptoms	
3	3	17	200	15.48	Much discomfort; retired in 10 min	155
			400	–	Marked symptoms; retired in 16 min	
			1,200	–	Light symptoms after 3 min	

Note: Concentration at 2,500 yd was 4.95 mg/cu m. At 3,000 yd, observers put on respirators after a few minutes. Slight effects were felt in a village 7 to 8 mi away.

* Calculated in Toxicology Division, Edgewood Arsenal, 1965.

3. CRDL Experiments (1958).

More recently, Gongwer and coworkers[10] and Punte and coworkers[11] reported the following tolerance times for men exposed to DM aerosols dispersed as preground powders.

Exposure concn mg/cu m	Tolerance* time sec
4	>180 (4)**
6	150, >180 (2)
7	168, >180
8	165, >180
12	174
13	150, 154
15	>180 (2)
16	>180 (2)
17	102
22	>120
25	>180
30	>120
33	>180
77	>120
92	92

These experiments[10, 11] indicated that concentrations ranging from 5 to 100 mg/cu m could not be tolerated during a 2- to 3-min exposure period by some subjects.

D. Lethality.

There have been thousands of human exposures to DM. Except in a few isolated cases, the men have survived and recovered without known aftereffects.. A few deaths have occurred.

---

* Where times are marked >, the exposures were terminated by the investigator.

** Number in parentheses indicates number of volunteers driven from exposure atmosphere.

E. Summary of Effects in Man.

The studies performed by Gongwer and Punte in 1958[10, 11] led to median incapacitating dose (ICt50) estimates of 10 to 350 mg/cu m for a 0.5- to 2.0-min exposure period. Since nausea, headache, and other systemic effects were noticed at Ct's of about 100 mg min/cu m and in view of liver damage noted in mice at Ct's of 4,000 to 6,000 mg min/cu m, it was deemed inadvisable to increase the dosage in human exposures. These experiments[10, 11] also indicated that the ICt50 for systemic effects is not less than 100 mg min/cu m, because at lower Ct's, none of the volunteers vomited or became nauseated.

Studies performed in 1957 by Wilding and coworkers* revealed that although the men showed resistance and tolerance to greater concentrations for longer times than those noted by Lawson and Temple (60 min at 0.14 mg/cu m),[6] the agent was detectable (had irritant effects) at very low concentrations. Men could detect DM after 5 to 20 min at concentrations of 0.03 to 0.15 mg/cu m. At 0.2 mg/cu m and above, its presence was usually noted in 5 min and often immediately. The latter value is in agreement with that reported in 1918 by Sherwood and Gavin (cited in Craighill and Folkhoff[9]), who gave 0.38 mg/cu m as the lowest concentration that is irritating to the throat.

The smoothed curve developed by Lawson and Temple[6] for intolerable concentrations of DM in man included concentrations of 22.3, 0.72, 0.23, and 0.14 mg/cu m for exposures of 1, 5, 15, and 60 min, respectively. It is likely that the ICt50's of 22 mg min/cu m for a 1-min exposure and 8 mg min/cu m for a 60-min exposure reported elsewhere[14] were derived from the curve by Lawson and Temple.

The results of field tests, shown in tables V and VI, indicate that some observers tolerated Ct's of DM near 100 mg min/cu m. Table V discloses that three unprotected observers tolerated Ct's of 83, 124, and 149 mg min/cu m, respectively. Table VI shows the responses of three men exposed to Ct's of 35, 50, and 155 mg min/cu m, respectively.

The ICt50 of 22.3 mg min/cu m for a 1-min exposure, as estimated by Lawson and Temple, is in disagreement with the experiments performed in 1958 by Gongwer and Punte.[10, 11] The earlier study[6] showed that men could tolerate concentrations from 22 to 92 mg/cu m for 1 min or more. In the latter tests,[10, 11] the men were told to resist the agent, and the airborne

* Wilding, J. L., et al. Aerosol Branch. 1957. Unpublished data.

concentrations were determined chemically. In the early study, the men were told to terminate the test when there was a feeling of distress and not to fight to the last limit of endurance. The airborne concentrations were estimated nominally in this study. (The quantities of agent in the exposure atmosphere were calculated from the amount of material disseminated in a given area). No chemical analysis was performed.

An important consideration concerning DM is its persistent incapacitating effects. The effects usually referred to are malaise, mental depression, nausea, and vomiting. In the experiments performed in 1958,[10,11] systemic effects, such as nausea and vomiting, were seen infrequently. Of 25 subjects exposed to Ct's ranging from 5 to 144 mg min/cu m, only two became nauseated. They were exposed to Ct's of 18 and 22 mg min/cu m. A similar indication is seen in the older data,[9] which shows that nausea was produced in three of 21 men exposed to a concentration of 2 mg/cu m for 140 sec to 15 min (Ct's of 4.6 to 30 mg min/cu m) and in two of 23 men exposed to 5 mg/cu m for periods of 45 sec to 12-1/2 min (Ct's of 3.75 to 62.5 mg min/cu m). The immediate effects given by Lawson and Temple indicate that a low frequency of systemic effects occurred in their experiments.

A summation of the available data indicates that the ICt50 for systemic effects has not been achieved in human exposures.

The safety factor for inhaled DM, based on the relationship between an LCt50 derived from animal data and the ICt50's for intolerable irritation and systemic effects, is discussed in section IV.

III. EFFECTS IN ANIMALS.

Appendix A describes in detail the experimental methods used by the American University (1918), Hazleton Laboratory (1963), and the Aerosol Branch, Toxicology Division, CRDL (1957 to 1965) for the determination of the inhalation toxicities of DM dispersed by laboratory methods or from thermal munitions. The reports from these laboratories include descriptions of the following: (1) materials—agent used for laboratory dispersions and test munitions, (2) animals, (3) exposure techniques, (4) particle-size determinations, (5) chemistry and bioassessment of DM, (6) animal observations, and (7) pathological studies.

A. Laboratory Toxicity Studies.

Laboratory No. 1 - War Department, Chemical Warfare Service, Research Division, American University Experimental Station, Washington, D. C.; investigators, Ransom and Bogart; 1918.

These experiments were performed with pure DM disseminated by dropping solutions of the material onto a heated surface. Only dogs were exposed during these studies.

The following observations are quoted from Ransom and Bogart.[4]

> Signs During Exposure - In dogs exposed to Cts of from 33,000 to 30,300 mg min/cu m there was immediate irritation of nose, eyes, but in only 5 of the 26 was there any sneezing. Possibly the most striking finding during the exposures was a delayed excitement. In paractically all cases, the animals were quiet or only moderately active during the first 5- to 15-minute exposure. This inactive period was followed by a sudden and prolonged period of excitement. The animals became frantic, and struggled furiously to get out of the box. During this excitement there was vomiting, retching, and defecation in practically every case. Marked salivation and lachrymation was also present.
>
> Signs After Exposure - There was always marked depression in the animals exposed to 0.53 mgm/liter or more. Lachrymation, salivation, and purulent conjunctivitis were also present, in most of the animals. Emaciation was common in all but the 4 animals exposed to the lower concentrations. There was nothing noteworthy in the symptoms before death. of the animals dying acutely.

Laboratory No. 2 - Hazleton Laboratory, Falls Church, Virginia; investigators, J. Mennear, H. Jennings, D. McCarthy, H. Bolden, J. Ott, B. Smith, and P. Warman; September 1963.

The following is quoted from Hazleton Laboratories Contract Report, September 1963.[15]

> The usual toxic manifestations following exposure to irritants, included lacrimation, ptosis, piloerection, nasal discharge (blood preceding expiration), frothing, salivation, urticaria, emesis, general depression, and decreased activity, dyspnea, hypernea, apnea, wheezing, tachycardia, anorexia, ataxia, asthenia, excessive urination and defecation, diarrhea, and prostration were observed.

Dogs exposed to DM (Ct's of from 1,610 to 64,200 mg min/cu m) exhibited a severe hind limb ataxia. Also, 3 of the dogs exposed to the highest dosage levels were comatose upon removal from the exposure chamber and were dead within several hours.

The toxic effects seen in the monkeys exposed over the same Ct range as the dogs, seemed to be less severe. The effects peculiar to monkeys were palpebral and penile edema.

Laboratory No. 3 - Aerosol Branch, Toxicology Division, Directorate of Medical Research, CRDL, Edgewood Arsenal, Maryland; investigators, J. T. Weimer, T. A. Ballard, W. E. Hickman, and C. L. Punte; 1957 to 1964.

These experiments were performed using pure DM disseminated either as a dry dust or sprayed in an acetone solution.

The following is quoted verbatim.[16]

Immediately upon exposure, the animals (rats, mice, and guinea pigs), exposed to DM concentrations varying from 11 to 2,940 mg/cu m, were hyperactive. Within a minute, nasal and ocular irritation were evident at all dosages. After several minutes of exposure, lacrimation and salivation were observed. After 5 to 15 minutes, the excitement was generally supplanted by lethargy and labored breathing. The latter signs often persisted for an hour or two after exposure. The other signs usually subsided within 5 to 10 minutes.

Laboratory No. 3 - Investigators, J. T. Weimer, R. L. Farrand, T. A. Ballard, T. E. Hess, G. F. Egan, C. F. Hoffman, J. W. Hiddeman, W. U. Thomas, G. L. Sell, J. S. Olson, R. P. Merkey, J. Burns, and W. M. Lawson; 1965.

1. Signs From Acute Exposures.

In these studies, rats, guinea pigs, rabbits, dogs, monkeys, and swine were exposed acutely to DM aerosols disseminated by various methods. The responses observed in these species followed the same pattern whether exposure was to pure DM disseminated from 10% acetone solutions or to DM

disseminated from the M6A1 or No. 113 Federal Laboratories* thermal grenades. The signs produced by exposures to the three systems of DM disseminated were very similar. Based on these observations, the three systems are treated as an entity. The times to onset of clinical signs and their duration are shown in table VII. A résumé of the responses observed in the seven species tested follows.

a. Rats and Guinea Pigs.

Signs occurring during exposure were irregular respiration, hyperactivity, and death. Postexposure signs were gasping, hypoactivity, decreased consumption of food or water for about 7 to 10 days, loss of weight, piloerection, and loss of fur.

Upon death, all animals appeared extremely dehydrated. Survivors began to appear normal after 14 days.

b. Rabbits.

During exposure, ocular and nasal irritation, lacrimation, rhinorrhea, respiratory difficulty, hyperactivity, squealing, convulsions, and death were seen. After exposure, survivors became hypoactive, exhibited eyelid ptosis, and developed conjunctivitis. After 7 days, loss of fur was noted in a large number of the animals. Loss of appetite was a precursor of death, but all animals continued to drink water. Convulsions, in most cases, were followed by death.

c. Dogs.

Immediately upon exposure, the dogs became extremely restless and jumped and barked. Salivation, retching, and vomiting occurred. The animals appeared intoxicated and became very unstable or ataxic to the extent that they actually fell and had difficulty standing. They had difficulty keeping their eyes open.

Upon removal from the chamber, the dogs were hypoactive and pawed at their faces. Gagging and vomiting persisted for about 24 hr. They consumed little or no food or water until about 7 days after exposure and were dehydrated and constipated. After 7 days, the animals appeared to be normal and resumed eating and drinking. There was a definite weight loss. Retching persisted throughout the observation period. Most deaths occurred in the first 7 days.

* Federal Laboratories, Inc., Saltsburg, Pennsylvania.

Table VII. Clinical Signs in Order of Appearance in Animals Inhaling DM Disseminated From a 10% Acetone Spray, the M6A1 Grenade, or the No. 113 Grenade

Signs	Times to response						
	Dog	Swine	Goat	Monkey	Rabbit	Rat	Guinea pig
			A. During Exposure, min				
Ocular and nasal irritation	I*	I	I	I	I	I	I
Lacrimation	1 - 3	1 - 3	1 - 3	1 - 3	1 - 3	1 - 3	1 - 3
Salivation	1 - 3	1 - 3	1 - 3	1 - 3	1 - 3	1 - 3	1 - 3
Gagging	1 - 3	1 - 3	1 - 3	1 - 3	1 - 3	1 - 3	1 - 3
Rhinorrhea	3 - 5	3 - 5	3 - 5	3 - 5	3 - 5	3 - 5	3 - 5
Hyperactivity	5 - 7	5 - 7	5 - 7	5 - 7	5 - 7	5 - 7	5 - 7
Regurgitation	10 - 15	10 - 15	—	10 - 15	—	—	—
Bloating	—	—	15	—	—	—	—
Respiratory difficulty	10 - 15	10 - 15	10 - 15	10 - 15	10 - 15	10 - 15	10 - 15
			B. Following Exposure, days				
Dyspenia	1 - 14	1 - 7	1 - 7	1 - 14	1 - 7	1 - 7	1 - 7
Gagging	1 - 30	—	—	1 - 30	—	1 - 14	1 - 14
Rhinorrhea	1 - 7	1 - 7	1 - 7	1 - 7	1 - 7	1 - 7	1 - 7
Conjunctivitis	—	—	1 - 7	—	1 - 14	—	—
			C. Following Exposure, hr				
Death	5 - 356	3 - 672	3 - 720	3 - 331	1 - 675	2 - 696	2 - 597

Note: Exposure Ct's varied from 1,100 to 61,000, 5,540 to 88,000, and 8,500 to 97,800 mg min/cu m, respectively, for the species exposed to DM disseminated from acetone sprays, M6A1 grenades, and No. 113 Federal Laboratories grenades.

* I = immediately.

d. Monkeys.

During the exposure, the following signs were noted: salivation, vomiting, respiratory difficulty, ataxia, and rhinorrhea. Upon removal from the chamber, the animals wheezed, exhibited ptosis, and were lethargic. Coughing and vomiting persisted for about 24 to 48 hr. Open breaks in the skin around the eyes and face were noted, possibly due to the agent or to pawing by the animals. Prior to death, the animals were face down and motionless; their breathing appeared to be depressed.

e. Goats.

Signs during exposure were hyperactivity shaking the head, rearing on the hindlegs, licking, chewing, frothing at the mouth, ataxia, convulsions, bloating, and death. For 7 days after exposure, the survivors were hypoactive, knelt on their forelegs, gagged, and vomited. The animals showed rhinorrhea, loss of weight, and generalized weakness. They knelt over and convulsed prior to death. All animals were bloated upon death.

f. Swine.

The signs seen during exposure were salivation, frothing at the mouth, ataxia, and irregular respiration. During the 14 days after exposure, the pigs had respiratory difficulty; they lost weight, and were dehydrated.

2. Pharmacology.

A joint program was initiated between the Basic Toxicology and Aerosol Branches of the Toxicology Department to determine the pharmacologic action of inhaled DM aerosols in dogs. The effects on the respiratory and cardiovascular systems were determined by J. E. Vestweber, R. K. Biskup, H. L. Snodgrass, R. L. Farrand, J. T. Weimer, and J. W. Hiddemen, Aerosol and Basic Toxicology Branches, Toxicology Department, Medical Research Laboratory.

a. Methodology.

Beagle dogs weighing 20 to 27 lb were anesthetized by intravenous administration of sodium pentobarbital. Continuous measurements were made of intracarotid blood pressure (by direct cannulation), arterial oxygen content (by a constant recording oximeter), respiratory rate and depth (by a plethysmograph) and intrathoracic blood pressure (by cannulation through the internal jugular vein); continuous electrocardiogram recordings were also made.

DM aerosols were dispersed from an acetone solution into a chamber. In two experiments, the dogs' muzzles were inserted directly into the chamber. The Ct's were 17,000 and 28,000 mg min/cu m. One dog receiving the lower dose died in 2 hr, and two others were sacrificed after 5 hr. In another test, the aerosol was breathed from the chamber through a cannula directly into the trachea. This animal received a Ct of 22,000 mg min/cu m. Death occurred 56 min from the start of the exposure.

One anesthetized and one unanesthetized dog were exposed to acetone vapors alone to furnish control data for animals exposed to the DM acetone spray. No toxic effects were seen in either animal during a 30-day postexposure observation period.

b. Results.

The percent increase or decrease (as related to control values) in the above-mentioned measurements for one dog surviving an inhalation exposure of 28,000 mg min/cu m and for one that died following an exposure of 17,000 mg min/cu m is shown in figure 1. The progressive pharmacological effects produced in one dog exposed by endotracheal administration to a concentration of DM aerosol of 287 mg/cu m for 56 min are shown in figure 2. Despite the use of heparin, accurate measurement of the arterial oxygen in the surviving dog was difficult because of clotting (figure 1).

3. Local Effects of Topically Applied DM on Eyes and Skin in Animals.

Local effects of topically applied DM on eyes and skin have been reported by Loevenhart,[17] Punte and coworkers,[16] and by the Aerosol Branch, Edgewood Arsenal, 1965.

Loevenhart stated that weak alcoholic solutions of DM applied to the skin of dogs caused slight hyperemia, with petechial hemorrhages and slight edema. After 7 days, a scab covered the area.

Punte and coworkers stated that DM doses of 0.5 and 1.0 mg in the eyes of rabbits caused immediately lacrimation and conjunctivitis. No permanent eye damage occurred.

The study of ocular and cutaneous effects of DM (suspended in corn oil) by the Aerosol Branch is reported as follows.

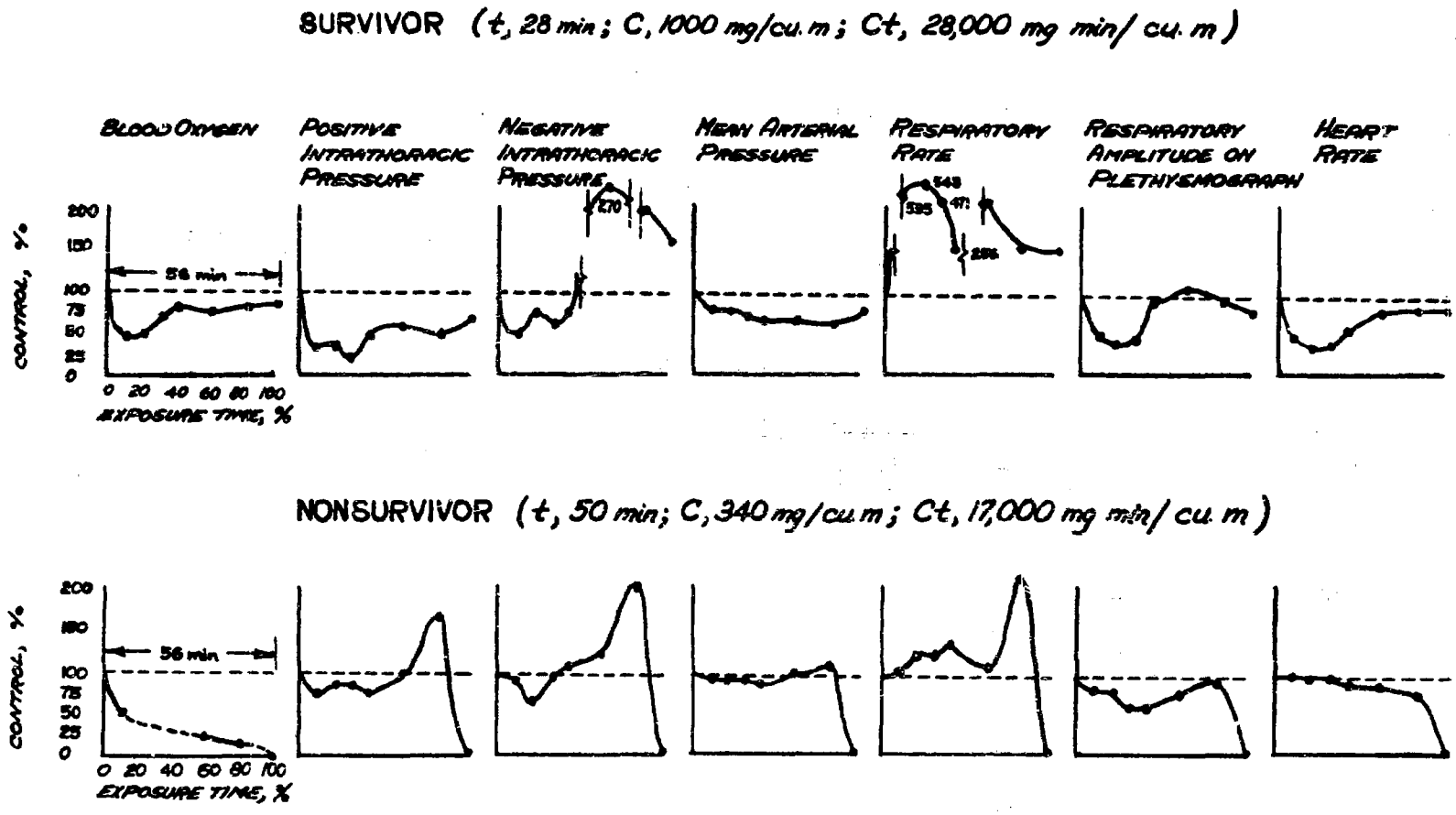

Figure 1. Pharmacological Effects Measured in a Dog That Survived and a Dog That Died From the Inhalation of DM Aerosols

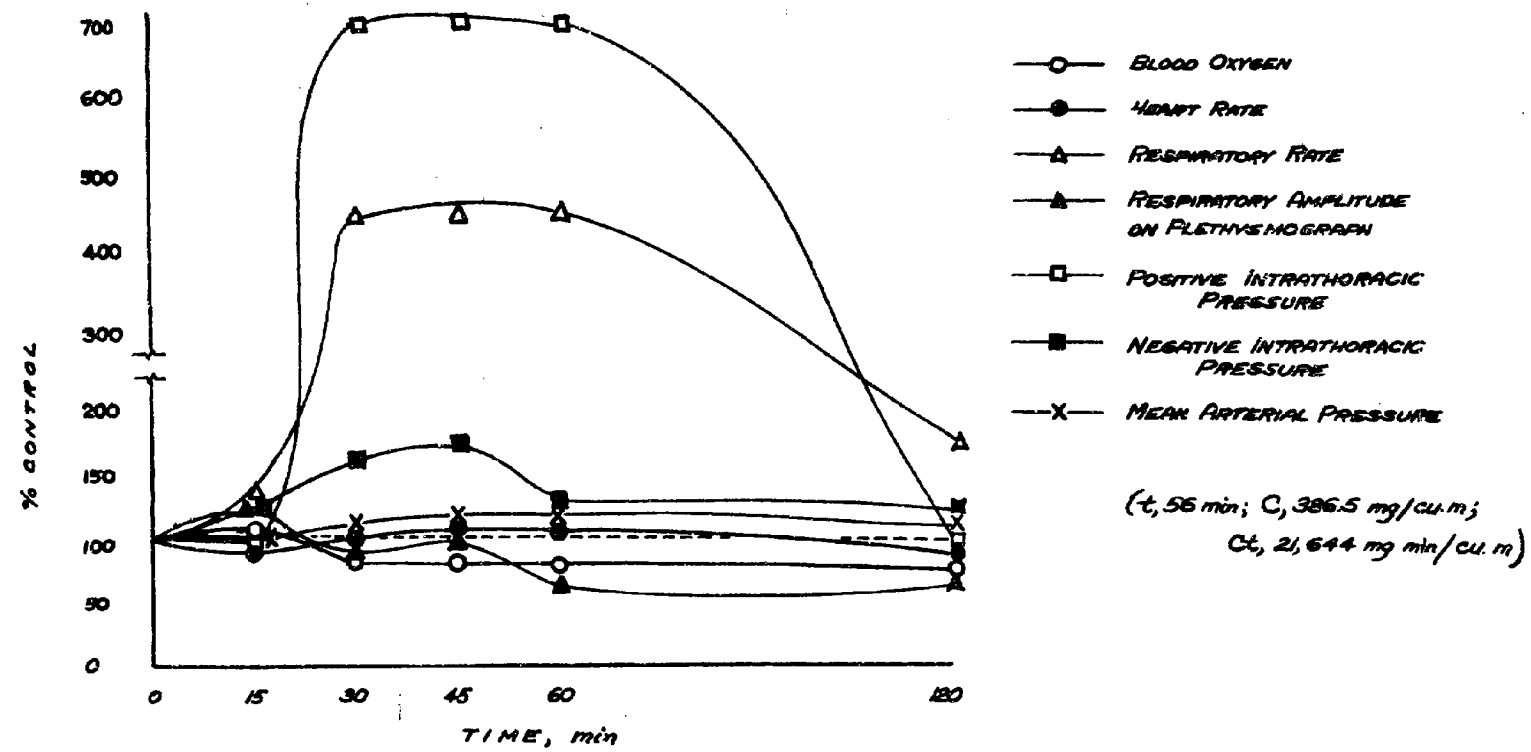

Figure 2. Pharmacological Effects Produced by Endotracheal Administration of a DM Aerosol to a Dog

a. Ocular Application; Investigator, W. U. Thomas.

A DM suspension in corn oil was administered ocularly to groups of six rabbits each at dose levels of 0.1, 0.2, 0.5, 1.0, and 5.0 mg. All animals were observed for 8 to 14 days. A dose of 0.1 mg produced no observable signs; 0.2 mg produced transitory and mild conjunctivitis; 0.5 mg produced transitory conjunctivitis and mild blepharitis; 1.0 and 5.0 mg produced permanent corneal opacity. Details of this study are shown in table VIII.

b. Cutaneous Application.

A DM suspension in corn oil was placed on the clipped banks of rabbits. Doses of 1.0, 10.0, 50.0, 75.0, and 100 mg were applied to groups of six rabbits each. Doses of 10 mg and higher produced necrosis. Details of this study are presented in table IX.

4. Lethality.

One of the most striking features of the acute inhalation toxicity of DM in animals is the marked variation in results of different experiments. In the early experiments performed during World War I, these discrepancies in results were sometimes attributed to erroneous estimations of airborne concentrations. Frequently, nominal estimations, rather than chemical analyses, were used. The British Red Book[12] declined to quote the toxicities in dogs, rabbits, monkeys, goats, etc., that had been reported in US Monogram 17, because these results were inconsistent and there was no reason to believe the nominal Ct's were valid.

In 1918, Gilbert[18] reported on the inhalation toxicity of DM in mice. He concluded that its lethal concentration was above 3,000 mg/cu m for a 10-min exposure. Nominal concentrations were used in the tests, and there was no consistent relationship between dose and number of deaths. A later summary[5] pointed out that the data did not justify this estimate. At one time, 30,000 mg min/cu m was used as an LCt50 value[15]; the value was possibly based on this earlier experiment in mice. The toxicity results of more recent analytically controlled experiments are as markedly variable as those of the early tests.

The results of the acute inhalation studies performed at the American University Experimental Station in 1918, the Hazleton Laboratories in 1963 to 1964, and the Aerosol Branch, Toxicology, Department Medical Research Laboratory, Research Laboratories, from 1957 to 1965 are shown in tables X to XV.

Table VIII. Eye Effects of Corn Oil Suspension of DM in Albino Rabbits

Rabbit No.	Signs observed beginning 24 hr after DM application*							
	1	2	3	6	7	10	12	14
	days				days			
	A. Dose, 0.1 mg							
1 - 6	N	N	N	N	N	N	N	N
	B. Dose, 0.2 mg							
7	C-	N	N	N	N	N	N	N
8	N	N	N	N	N	N	N	N
9	C-	N	N	N	N	N	N	N
10	N	N	N	N	N	N	N	N
11	C-	N	N	N	N	N	N	N
12	N	N	N	N	N	N	N	N
	C. Dose, 0.5 mg							
13	C, B-	C-, B-	C-	N	N	N	N	N
14	C, B-	N	N	N	N	N	N	N
15	C, B-	C-	N	N	N	N	N	N
16	C, B-	C	N	N	N	N	N	N
17	C-	N	N	N	N	N	N	N
18	C-, B-	N	N	N	N	N	N	N
	D. Dose, 1.0 mg							
19	C+	C	C-	N	N	N	N	N
20	C	C-	C-	N	N	N	N	N
21	CO, C, B+, S	CO, C, B+, S	CO, C+, B+, S	CO, B+, C+, S	CO, C, B	CO	CO	CO
22	CO, C, B+, S	C	C-	N	N	N	N	N
23	CO, C+, B	CO, C	C-	N	N	N	N	N
24	CO, C, B, S	C, B-, S	C-	N	N	N	N	N
	E. Dose, 5.0 mg							
25	CO, B, C	CO, C, B-	CO, C	CO, C	CO	N	N	N
26	CO, C, B, S	CO, C, B-	CO, C	CO, C-	CO	N	N	N
27	CO, C, B	CO, C, B+, S	CO, C, B, S	CO, C, B, S	CO, C, B	CO, C-, B-	CO	CO**
28	CO, C, B	CO, C, B, S	CO, C+, B+	CO, C+, B	CO, C, B	CO, B-	CO	CO**
29	CO, C, B	CO, B, C	C-	C-	C-	N	N	N
30	CO, C, B	CO, C, B, S	CO, C+, B+, S	CO, C, B, S	CO, C, B	CO, C, B	CO, C, B	CO**

* Signs: C = conjunctivitis, moderate; C-, mild; C+, severe.
B = blepharitis, moderate; B-, mild; B+, severe.
CO = corneal opacity.
S = swelling.
N = normal.

** Severe eye damage.

Table IX. Cutaneous Effects of 100-mg/ml Corn Oil Suspension of DM in Clipped Albino Rabbits

Rabbit No.	Signs observed beginning 24 hr after DM application*						
	1	2	3	4	7	9	10
	days						
A. Dose, 1 mg; Amount Applied, 0.01 ml							
1	N	N	N	N	N	N	N
2	N	N	N	N	N	N	N
3	N	N	N	N	N	N	N
4	N	N	N	N	N	N	N
5	N	N	N	N	N	N	N
6	N	N	N	N	N	N	N
B. Dose, 10 mg; Amount Applied, 0.1 ml							
7	E-	E-	E-	E-	E-	Nec-	Nec-
8	E-	E-	E-	E-	E-	E-	E-
9	E-	Nec-	Nec-	E, Nec-	E, Nec-	Nec-	Nec-
10	E-	E-	E-	E-	E-	E-	E-
11	E-	E-	E-	E-	E-	E-	E-
12	E-	E-	E-	E-	E-	E-	E-
C. Dose, 50 mg; Amount Applied, 0.5 ml							
13	E	E	E	E	E	E-	E-
14	E	E	E	Nec	Nec	Nec	Nec
15	E	E	E	Nec	Nec	Nec	Nec
16	E	E	E	Nec	Nec	Nec	Nec
17	E	E	E	Nec	Nec	Nec	Nec
18	E	E	E	Nec	Nec	Nec	Nec
D. Dose, 75 mg; Amount Applied, 0.75 ml							
19	E	Nec	Nec	Nec	Nec	Nec	Nec
20	E	Nec	Nec	Nec	Nec	Nec	Nec
21	E	Nec	Nec	Nec	Nec	Nec	Nec
22	E	Nec	Nec	Nec	Nec	Nec	Nec
23	E	Nec	Nec	Nec	Nec	Nec	Nec
24	E	Nec	Nec	Nec	Nec	Nec	Nec
E. Dose, 100 mg; Amount Applied, 1.0 ml							
25	E	E	E	E+, Nec	E, Nec+	E, Nec+	E, Nec+
26	E	E	E-, Nec	E+, Nec+	E, Nec+	E, Nec+	E, Nec+
27	E	E	E-, Nec	E+, Nec+	E, Nec-	E, Nec+	E, Nec+
28	E	E	E-, Nec	E+, Nec+	E, Nec	E, Nec+	E, Nec+
29	E	E	E-, Nec	E+, Nec+	E, Nec+	E, Nec+	E, Nec+
30	E	E	E-, Nec	E+, Nec+	E, Nec+	E, Nec+	E, Nec+

* Signs: E = erythema, moderate; E-, mild; E+, severe.
Nec = necrosis, moderate; Nec-, mild; Nec+, severe.
N = normal.

Table X. Inhalation Toxicity Data for Pure DM and a Bliss Statistical Analysis of the Data for Each Experiment in Each Species of Animals

Pertinent information	Ct	Concn	Exposure time	Observation period	Mortality fraction	Times to death	Bliss statistical analysis				
							P	ED (P)	Lower limit	Upper limit	Slope
	mg min/cu m	mg/cu m	min	days				mg min/cu m			
A. Rat											
Source: Aerosol Branch, Director of Medical Research, Edgewood Arsenal	540	181	30	14	2/6	–*	1	3,901	3,159	4,817	13.2
	13,200	440	30	14	6/6	–	16	4,921	3,885	6,234	
Dates: June - Sept 1957	13,250	442	30	14	6/6	–	30	5,342	4,137	6,898	
Investigator: T. A. Ballard	15,660	522	30	14	6/6	–	50	5,854	4,582	7,479	
	15,950	532	30	14	6/6	–	84	6,963	5,550	7,401	
Method of dispersion: dust							99	8,783	5,841	13,209	
Source: same	2,004	167	12	14	0/10	–	1	2,051	815	5,163	4.0
Date: Jan 1964	4,136	188	22	14	1/10	–	16	4,346	2,908	6,493	
Investigator: W. E. Hickman	5,460	182	30	14	4/10	–	30	5,665	4,377	7,331	
	7,363	199	37	14	4/10	–	50	7,614	6,351	9,579	
Method of dispersion: spray (in acetone)	8,250	165	50	14	6/10	–	84	13,340	7,765	22,917	
	10,800	108	100	14	7/10	–	99	28,263	9,627	83,006	
Source: same	569	–	–	14	0/10	–	1	133	7	2,525	2.4
Date: Sept 1963	1,122	–	–	14	5/10	–	16	487	103	2,294	
Investigators: T. A. Ballard and W. E. Hickman	2,385	–	–	14	8/10	–	30	770	263	2,253	
	4,069	–	–	14	6/10	–	50	1,285	710	2,326	
	5,823	–	–	14	10/10	–	84	3,394	1,610	7,152	
Method of dispersion: same	7,423	–	–	14	10/10	–	99	12,454	1,543	100,498	
Source: same	300	–	–	14	0/10	–	1	290	8	10,704	2.4
Date: Aug 1963	1,030	–	–	14	2/10	–	16	1,036	390	2,750	
Investigator: W. E. Hickman	2,255	–	–	14	4/10	–	30	1,623	835	3,153	
							50	2,678	613	11,706	
Method of dispersion: same							84	6,923	212	–	
							99	24,716	47	–	
Source: same	2,075	208	10	14	0/10	–	1				
Date: Apr 1963	4,155	208	20	14	0/10	–					
Investigator and method of dispersion: same	6,000	240	25	14	0/10	–					
	8,096	245	33	14	0/10	–					
	10,124	260	39	14	0/10	–					
	12,242	395	31	14	0/10	–					
Source, investigator, and method of dispersion: same	2,832	354	8	14	0/10	–	1	23,704	15,076	37,270	14.5
	7,292	561	13	14	0/10	–	16	28,271	27,531	31,120	
Date: May 1963	11,720	617	19	14	0/10	–	30	31,533	28,316	35,116	
	21,410	824	26	14	0/10	–	50	34,264	29,726	39,495	
	27,810	927	30	14	1/10	–	84	40,109	25,711	62,570	
							99	49,528	21,764	112,708	
Source, investigator, and method of dispersion: same	612	–	–	14	0/10	–	1	232	4	14,068	2.3
	792	–	–	14	2/10	–	16	891	234	3,378	
Date: Aug 1963	1,808	–	–	14	0/18	–	30	1,432	891	2,301	
	2,480	–	–	14	1/10	–	50	2,431	987	5,989	
	3,560	–	–	14	10/10	–	84	6,631	351	125,401	
							99	25,424	82	7,972,664	
Source: same	2,149	72	30	14	5/6	–	1	61	0.0	7,721,281	1.7
Date: 1957	4,388	293	15	14	3/6	–	16	376	0.3	412,191	
Investigator: T. A. Ballard	6,600	220	30	14	6/6	–	30	712	3	145,528	
	6,960	232	30	14	4/6	–	50	1,454	47	44,912	
Method of dispersion: dust	10,080	336	30	14	6/6	–	84	5,627	2,667	11,869	
	18,700	623	30	14	6/6	–	99	34,465	318	3,737,671	
	19,200	640	30	14	6/6	–					

* Dashes indicate data not recorded.

Table X. (contd)

Pertinent information	Ct	Concn	Exposure time	Observation period	Mortality fraction	Times to death	Bliss statistical analysis				
							P	ED (P)	Lower limit	Upper limit	Slope
	mg min/cu m	mg/cu m	min	days				mg min/cu m			
B. Mouse											
Source: same	2,004	120	20	14	0/10	–	1	1,861	1,193	2,902	5.0
Date: Feb 1964	4,136	188	22	14	1/10	15 - 20 min	16	3,431	2,762	4,262	
	5,460	182	30	14	4/10	15 - 30 min	30	4,257	3,639	4,980	
Investigator: W. E. Hickman	7,363	199	37	14	4/10	10 - 15 min (3),*	50	5,417	4,732	6,201	
	8,250	165	50	14	6/10	45 (1)	84	8,552	6,683	10,943	
Method of dispersion: spray (in acetone)	10,800	108	100	14	7/10	15 - 30 min (3), ON** (3) <30 min (7)	99	15,768	9,766	25,456	
Source: same	315	11	30	14	0/6	–	1	6	0	–	0.5
Date: June 1957	2,150	143	15	14	2/6	–	16	1,615	0.0	246,890,910	
	15,780	526	30	14	3/6	–	30	11,814	663	210,551	
Investigator: T. A. Ballard	16,650	1,110	15	14	1/6	–	50	108,715	197	29,868,752	
	53,150	1,772	30	14	2/6	–	84	7,316,343	0	–	
Method of dispersion: dust	55,320	3,688	15	14	0/6	–	99	205,256,510	0	–	
	60,500	2,420	25	14	4/6	–					
	70,000	2,333	30	14	5/6	–					
	84,990	2,833	30	14	2/6	–					
C. Guinea Pig											
Source: same	3,800	127	30	14	0/4	–	1	1,397	263	7,412	2.4
Date: July - Sept 1957	4,550	152	30	14	0/4	–	16	4,996	2,125	11,746	
	5,900	197	30	14	2/4	–	30	7,833	4,297	14,272	
Investigator and method of dispersion: same	11,600	387	30	14	2/4	–	50	12,936	8,616	19,421	
	13,000	433	30	14	3/4	–	84	33,498	17,429	64,380	
	16,400	547	30	14	3/4	–	99	119,779	28,381	505,509	
	18,200	607	30	14	2/4	–					
	19,400	647	30	14	2/4	–					
	25,300	843	30	14	1/4	–					
	27,400	913	30	14	3/4	–					
	29,640	988	30	14	4/4	–					
	37,800	1,260	30	14	4/4	–					
	85,200	2,840	30	14	4/4	–					
Source: same	1,350	135	10	14	2/10	2 - 3 days	1	450	52	3,936	2.2
Date: Feb 1964	2,475	124	20	14	2/10		16	1,766	766	4,072	
	3,225	161	20	14	2/10		30	2,861	1,724	4,748	
Investigator: W. E. Hickman	8,875	178	50	14	8/10		50	4,900	2,684	8,946	
							84	13,596	2,988	61,853	
Method of dispersion: spray (in acetone)							99	53,322	2,921	973,507	
Source: same	569	–	–	14	0/10	–	1	160	0.0	1,649,895	1.1
Date: Sept 1963	1,122	–	–	14	0/10	–	16	2,529	1,845	3,647	
	2,385	–	–	14	4/10	–	30	6,701	1,012	44,363	
Investigators: T. A. Ballard and W. E. Hickman	4,069	–	–	14	3/10	–	50	19,865	99	3,931,104	
	5,823	–	–	14	2/10	–	84	156,018	1	1,701,458,600	
Method of dispersion: same	7,423	–	–	14	2/10	–	99	2,465,079	0.0	–	
Source, date, investigators, and method of dispersion: same	1,041	–	–	14	5/10	–	1	1.2	0.0	1,526	0.8
	1,178	–	–	14	5/10	–	16	60	3	1,126	
	2,282	–	–	14	6/10	–	30	238	74	770	
							50	1,102	277	4,380	
							84	20,064	1,900	211,849	
							99	977,989	1,325	7,217,768,459	

* Number in parenthesis indicates number of mortalities at the *given times; otherwise, a* single mortality occurred.

** ON = overnight.

Table X. (contd)

Pertinent information	Ct	Concn	Exposure time	Observation period	Mortality fraction	Times to death	Bliss statistical analysis				
							P	ED (P)	Lower limit	Upper limit	Slope
	mg min/cu m	mg/cu m	min	days					mg min/cu m		
Source: same	2,075	208	10	14	2/10	24 hr	1	53	0.1	42,624	1.0
Date: Sept 1963	4,155	208	20	14	3/10		16	1,300	162	10,434	
Investigators: T. A. Ballard and W. E. Hickman	6,000	240	25	14	2/10		30	4,032	2,544	6,650	
	8,039	234	33	14	5/10		50	14,249	6,529	31,098	
	10,124	260	37	14	4/10		84	156,183	1,879	13,002,838	
Method of dispersion: spray (in acetone)	12,242	395	31	14	5/10		99	3,855,174	491	4,027,738,860	
				D. Dog							
Source: C. A. Ransom and F. B. Bogart	3,300	110	30		0/2	–	1	879	1.6	–	1.9
	4,200	140	30		0/2	–	16	4,237	219	82,048	
Date: 1918	9,000	300	30		2/2	48, 72 hr	30	7,582	1,197	45,541	
	12,000	400	30	14	1/2	12 days	50	13,718	6,320	29,776	
Method of dispersion: Molten alcoholic solution dropped on a hotplate	18,000	600	30	12, 14	2/4	50 hr, 14 days	84	44,393	4,447	–	
	21,000	700	30	12	1/2	10 days	99		764	–	
	24,000	800	30	12, 15	2/2	18, 48 hr					
	27,000	900	30		2/2	18, 18 hr					
	27,600	920	30		2/2	12, 48 hr					
	30,300	1,010	30	12	1/2	4 days					
Source: Aerosol Branch	9,955	524	19	14	0/2	–	1	5,160	2,916	9,132	3.8
Date: 16 Sept 1963	14,952	516	29	14	1/2	<24 hr	16	12,637	4,121	32,864	
Investigator: J. T. Weimer	24,956	734	34	14	1/2	<24 hr	30	15,506	5,139	46,791	
							50	21,358	7,272	62,727	
Method of dispersion: spray (in acetone)							84	39,200	20,531	74,845	
							99	88,400	22,532	346,821	
Source: Hazleton Laboratories	1,610	107	15	30	0/2	–	1	15,277	–	–	57.0
Date: 1 Sept 1963	14,400	480	30	30	0/2	–	16	16,121	–	–	
Investigators: J. Mennear et al.	19,500	433	45	30	2/2	2, 3 days	30	16,430	–	–	
	35,000	583	60	30	2/2	1, 3 days	50	16,782	–	–	
Method of dispersion: spray (in acetone)	64,200	433	125	30	2/2	1, 1 days	84	17,469	–	–	
							99	18,434	–	–	
Source: Aerosol Branch	5,100	–	–	14	0/8	–	1	5,285	1,276	21,920	4.1
Date: May 1964	8,517	–	–	14	1/8	–	16	11,266	6,292	20,269	
Investigators: J. T. Weimer and W. E. Hickman	16,680	–	–	14	2/8	–	30	14,716	10,034	21,584	
	25,480	–	–	14	6/8	–	50	19,927	13,157	29,880	
							84	34,895	13,427	90,687	
Method of dispersion: spray (in acetone)							99	74,393	11,928	463,958	
				E. Monkey (Rhesus)							
Source: Hazleton Laboratories	1,610	107	15	30	0/2	–	1				31.5
Date: 1 Sept 1963	14,400	480	30	30	0/2	–	6	24,266			
Investigators: J. Mennear et al.	19,500	483	45	30	0/2	–	30	25,115			
	35,000	583	60	30	2/2	11, 12 days	50	26,098			
Method of dispersion: spray (in acetone)	64,200	433	125	30	2/2	1, 1 days	84	28,067			
							99	30,939			
				F. Monkey (Squirrel)							
Source: Aerosol Branch	5,880	147	40	–	0/6	–	1	2,919	130	65,560	4.3
Date: May 1964	9,464	182	52	–	2/6	–	16	5,930	1,444	24,353	
Investigators: J. T. Weimer and W. E. Hickman	10,725	215	50	–	6/6	–	30	7,616	3,323	17,457	
	12,710	155	82	–	4/6	–	50	10,069	7,399	13,702	
	23,760	167	142	–	5/6	–	84	17,091	5,070	57,612	
Method of dispersion: same							99	34,726	1,891	–	

Table XI. A Bliss Statistical Analysis of Pure DM Toxicity for the Combined Mortalities of Each Species, All Rodents, All Nonrodents, and All Species Combined

(Experiments performed from 1918 to 1964)

Species or animal grouping	Bliss statistical analysis				
	P	ED(P)	Lower limit	Upper limit	Slope
		mg min/cu m			
Dogs	1	3,692	800	6,308	3.4
	16	9,088	4,650	12,151	
	30	12,491	8,120	15,914	
	50	17,809	13,700	23,732	
	84	34,898	25,623	73,010	
	99	85,912	49,040	397,347	
Mice	1	4	0.0	130	0.6
	16	861	0.4	3,299	
	30	5,659	382	15,555	
	50	46,244	16,617	3,801,791	
	84	2,484,742	222,979	12,252,150,000,000	
	99	515,630,850	5,084,766	—	
Rats	1	4.7	0.0	37	0.7
	16	347	77	1,008	
	30	2,307	1,067	3,664	
	50	14,045	8,473	36,383	
	84	431,659	109,391	11,149,252	
	99	42,393,054	2,867,994	27,927,439,000	
Guinea pigs	1	30	0.9	138	0.9
	16	836	222	1,567	
	30	2,690	1,381	4,123	
	50	9,906	6,420	20,093	
	84	117,363	43,998	1,089,164	
	99	3,215,971	479,828	276,097,340	
Monkeys	1	1,987	14	4,477	3.0
	16	5,498	581	8,540	
	30	7,874	2,037	11,288	
	50	11,756	6,686	19,023	
	84	25,140	16,531	197,278	
	99	69,567	31,776	7,907,435	
All rodents (mice, rats, and guinea pigs)	1	5	0.3	24	0.7
	16	204	178	927	
	30	2,597	1,598	3,686	
	50	16,179	10,996	26,929	
	84	519,644	180,456	3,402,042	
	99	54,136,036	6,795,347	2,268,730,400	
All nonrodents	1	2,537	821	4,268	3.0
	16	7,110	4,203	9,346	
	30	10,230	7,252	12,714	
	50	15,351	12,307	19,401	
	84	33,141	24,823	58,468	
	99	92,893	54,119	300,632	
All species combined	1	10	1	37	0.7
	16	669	303	1,111	
	30	2,915	1,957	3,935	
	50	15,052	11,041	22,941	
	84	338,579	148,643	1,283,210	
	99	21,893,306	4,314,795	314,790,270	

Note: All experiments were performed between 1918 and 1964, inclusively.

Table XII. Acute Inhalation Toxicity of DM Disseminated From a 10% Acetone Solution and a Bliss Statistical Analysis of the Mortality Responses

(Experiments performed in 1965)

Species	Ct	Concn	Exposure time	Mortality fraction	Times to death	Bliss statistical analysis				
						P	ED (P)	Lower limit	Upper limit	Slope
	mg min/cu m	mg/cu m	min		hr		mg min/cu m			
Monkey	40,000	296	135	6/6	28, 43, 149, 190(2),* 248	1	11,604	6,339	21,242	12.5
	25,085	214	117	6/6	43, 47, 67, 148, 235, 307	16	14,842	10,907	20,196	
	20,800	219	95	4/6	47, 65, 238, 286	30	16,189	13,038	20,101	
	16,720	209	80	3/6	192, 278, 350	50	17,837	15,351	20,725	
	12,555	279	45	0/6	—	84	21,434	16,740	27,445	
	5,940	297	20	0/6	—	99	27,416	16,050	46,828	
Dog	16,720	209	80	6/6	10, 16, 17, 35(3)	1	2,709	1,218	6,022	5.64
	12,555	279	45	4/6	18, 20, 42, 116	16	4,995	3,251	7,675	
	9,060	206	44	5/6	63, 86, 278, 336, 356	30	6,199	4,450	8,636	
	5,940	297	20	1/6	305	50	7,888	5,951	10,457	
	2,960	212	14	0/6	—	84	12,455	8,205	18,908	
						99	22,970	10,489	50,297	
Goat	41,600	210	198	6/6	4, 16(2), 72, 77, 113	1	3,631	990	13,316	4.41
	30,000	227	132	6/6	22(2), 71, 95, 240, 552	16	7,245	3,537	14,840	
	19,640	216	91	4/6	18, 90, 198	30	8,246	5,376	15,902	
	9,800	233	42	3/6	20(2), 239	50	12,135	8,051	18,292	
	5,062	230	22	0/5	—	84	20,327	12,010	34,401	
						99	40,556	13,986	117,603	
Swine	61,000	223	273	3/6	5.5, 20, 167	1	6,183	154	247,970	2.42
	41,600	210	198	2/6	4, 335	16	21,913	7,423	64,686	
	30,000	227	132	2/6	47(2)	30	34,245	19,928	58,847	
	19,640	216	91	1/6	42	50	56,364	16,709	190,140	
	9,900	206	48	0/6	—	84	114,930	6,141	3,420,500	
						99	513,700	1,473	—	
Rat	61,000	223	273	20/20	4, 8, 20(4), 47(5), 71, 95(2), 118(2), 134, 147(2) 168	1	12,296	8,708	17,364	11.96
	40,000	296	135	20/20	3(2), 47(2), 120(10), 190(4), 216(2)	16	15,887	13,582	18,584	
	25,085	214	117	18/20	29, 110(12), 134, 158, 211(3)	30	17,390	15,744	19,209	
	19,640	216	91	14/20	68(3), 140(3), 146, 148, 166(6)	50	19,237	17,924	20,646	
	16,720	209	80	1/20	11	84	23,290	19,644	27,614	
	12,555	279	45	1/20	21	99	30,092	21,000	43,120	
	5,940	297	20	0/20	—					
Guinea pig	16,720	209	80	16/20	11(6), 17, 35(7), 42, 64, 96	1	420	154	1,142	2.23
	12,555	279	45	19/20	19(14), 26(2), 528(2), 552	16	1,658	971	2,833	
	5,940	297	20	11/20	16(8), 21(2), 40	30	2,692	1,805	4,017	
	2,960	212	14	8/20	14, 16, 38(5), 70	50	4,623	3,391	6,303	
	1,100	220	5	1/20	230	84	12,885	8,252	20,119	
						99	50,840	20,849	123,970	
Rabbit	40,000	296	135	6/6	2, 25(6)	1	173	0.00	0.00	1.90
	34,560	300	115	6/6	2(5), 24	16	870	0.00	0.00	
	29,140	307	95	6/6	2(4), 2.5, 24	30	1,538	0.00	0.00	
	20,900	279	75	6/6	1.5, 2, 24(3), 48	50	2,903	0.00	0.00	
	11,070	246	45	4/6	1.2, 2, 24, 48	84	9,687	1,309	71,715	
	8,050	268	30	4/6	24(2), 48, 72	99	48,638	0.00	—	
	4,290	286	15	5/6	24, 72(2), 216, 240					
All rodents (rats and guinea pigs)						1	563	42	7,404	1.81
						16	3,079	931	10,175	
						30	5,609	2,733	11,512	
						50	10,951	8,397	14,282	
						84	38,947	15,269	99,344	
						99	213,003	21,053	2,150,884	
All nonrodents						1	217	4	11,719	1.39
						16	1,970	276	14,037	
						30	4,292	1,217	15,142	
						50	10,233	5,996	17,465	
						84	53,155	16,537	170,861	
						99	482,792	20,161	11,561,368	
All species combined						1	804	203	3,178	1.96
						16	3,834	2,009	7,316	
						30	6,653	4,463	9,919	
						50	12,306	10,283	14,726	
						84	39,498	23,804	65,539	
						99	188,289	55,973	647,746	

* Number in parenthesis number of mortalities at the given times; otherwise, a single mortality occurred.

Table XIII. A Bliss Statistical Analysis of Pure DM Toxicity for the Combined Mortalities of Each Species, All Rodents, All Nonrodents, and All Species Combined

(Experiments performed from 1918 to 1965)

Species or animal grouping	Bliss statistical analysis				
	P	ED (P)	Lower limit	Upper limit	Slope
		mg min/cu m			
Mice	1	4	0.0	130	0.6
	16	860	0.4	3,299	
	30	5,659	382	15,555	
	50	46,245	16,617	3,803,104	
	84	2,485,012	222,988	21,268,239,000	
Rats	1	50	9.6	138	1.0
	16	1,192	607	1,851	
	30	3,649	2,479	4,890	
	50	12,710	9,636	17,871	
	84	135,506	73,360	359,765	
	99	3,223,638	962,518	23,160,398	
Guinea pigs	1	99	23	236	1.3
	16	1,099	583	1,638	
	30	2,564	1,742	3,399	
	50	6,599	5,087	8,909	
	84	39,616	24,235	88,749	
	99	436,807	166,153	2,274,967	
Rabbits	1	173	0.0	1,420	1.9
	16	870	0.0	3,323	
	30	1,538	0.0	4,565	
	50	2,903	0.0	6,745	
	84	9,687	0.0	3,125,798	
	99	48,638	18,711	0.0	
Dogs	1	1,979	536	3,535	2.7
	16	6,052	3,306	8,212	
	30	8,980	6,060	11,463	
	50	13,945	10,857	18,249	
	84	32,130	23,200	62,325	
	99	98,261	53,580	386,501	
Monkeys	1	3,615	1,231	5,680	4.0
	16	7,811	4,556	10,081	
	30	10,252	7,092	12,649	
	50	13,886	10,984	17,235	
	84	24,685	19,429	40,165	
	99	53,340	34,699	149,876	
Goats	1	3,631	990	13,316	4.4
	16	7,245	3,537	14,840	
	30	9,246	5,376	15,902	
	50	12,135	8,051	18,292	
	84	20,327	12,010	34,401	
	99	40,556	13,986	117,603	
Swine	1	6,183	154	247,970	2.4
	16	21,913	7,423	64,686	
	30	34,245	19,928	58,847	
	50	56,364	16,709	190,140	
	84	114,930	6,141	3,420,500	
	99	513,700	1,473		
All rodents	1	33	9	77	0.9
	16	949	569	1,372	
	30	3,120	2,329	3,948	
	50	11,769	9,451	15,233	
	84	145,912	86,878	305,618	
	99	4,248,978	1,517,258	18,937,682	
All nonrodents	1	899	307	1,679	2.0
	16	4,201	2,491	5,780	
	30	7,238	5,119	9,113	
	50	13,280	10,800	16,030	
	84	41,983	31,769	65,542	
	99	196,093	110,122	528,632	
All species combined	1	57	22	113	1.0
	16	1,178	788	1,598	
	30	3,431	2,693	4,185	
	50	11,309	9,548	13,600	
	84	103,616	74,383	179,939	
	99	2,246,754	1,043,636	6,367,579	

Table XIV. Acute Inhalation Toxicity of DM Disseminated From an M6A1 Munition and a Bliss Statistical Analysis of the Mortality Responses

(30-Day observation; experiments performed in 1965)

Species	Ct	Concn	Exposure time	Mortality fraction	Times to death	Bliss statistical analysis				
						P	ED(P)	Lower limit	Upper limit	Slope
	mg min/cu m	mg/cu m	min		hr		mg min/cu m			
Monkey	36,500	2,808	13	4/6	17(3), *19	1	4,324	441	42,314	3.5
	34,900	2,685	13	6/6	18(4), 20, 192	16	10,263	3,420	29,912	
	24,200	2,689	9	3/6	22, 23(2)	30	13,923	7,169	27,041	
	17,600	2,514	7	4/6	24(2)	50	19,569	14,193	26,980	
	14,400	1,800	8	3/6	43(3)	84	37,302	15,593	89,236	
	13,900	1,986	7	0/6	–	99	88,538	11,119	–	
Dog	43,700	2,913	15	5/6	5, 22(3), 48	1	13,351	6,417	27,776	7.2
	36,900	2,460	15	5/6	24(5)	16	20,482	13,906	30,167	
	29,500	2,269	13	2/6	24, 91	30	23,821	17,878	31,739	
	17,600	2,514	7	1/6	41	50	28,193	22,673	35,212	
	14,300	1,586	9	0/6	–	84	38,802	27,857	54,049	
	6,200	886	7	0/6	–	99	59,529	30,599	115,812	
Goat	36,500	2,808	13	5/6	17(2), 19, 48, 72	1	368	0.1	–	1.7
	34,900	2,685	13	6/6	18(5), 138	16	2,156	16	–	
	25,600	2,327	11	4/6	3, 4(2), 50	30	4,025	112	–	
	14,400	1,800	8	4/6	44(2), 288, 360	50	8,076	945	69,016	
	12,200	2,033	6	4/6	44(3), 288	84	30,228	10,063	90,804	
						99	–	1,664	–	
Swine	62,700	2,508	25	5/6	16(2), 21, 40, 168	1	2,746	0.0	8,047,313,100	2.1
	45,700	2,688	17	4/6	17(4)	16	12,151	35.1	4,206,301	
	39,000	2,435	16	1/6	48	30	20,540	1,405	300,255	
	14,900	2,129	7	3/6	24(2), 42	50	36,011	12,202	111,530	
	13,900	1,986	7	0/6	–	84	111,116	49	255,955,870	
						99	495,520	0.0	9,640,477,700,000	
Rabbit	45,700	2,688	17	4/6	17(4)	1	2,292	0.0	4,522,860,000	1.9
	39,000	2,600	15	5/6	3, 24(4)	16	11,974	68.4	2,094,405	
	37,000	2,435	16	4/6	48, 65, 165(2)	30	21,464	3,443.1	135,812	
	34,900	2,653	13	1/6	18	50	41,159	84645.4	221,577	
	29,500	2,269	13	0/6	–	84	141,468	23.5	851,040,070	
	18,600	2,657	7	4/6	432(2), 456(2)	99	739,116	0.0	49,695,089,000,000	
Rat	88,000	2,588	34	14/20	1(6), 2(4), 24(3), 48	1	16,409	13,496	19,651	3.8
	80,000	2,666	30	15/20	1(4), 2(5), 24(5), 48	16	36,674	34,151	39,382	
	69,500	2,574	27	15/20	4(5), 24(9), 48	30	38,707	47,006	50,470	
	55,800	2,066	27	2/20	2, 48	50	66,856	64,033	69,804	
	42,100	2,216	19	1/20	96	84	121,852	106,944	–	
	36,500	2,808	12	0/20	–	99	–	–	–	
	34,900	2,685	13	5/20	259(5)					
	17,600	2,514	7	1/20	72					
	14,300	1,586	9	1/20	268					
Guinea pig	17,600	2,514	7	18/20	3(6), 24(12)	1	2,542	1,705	3,780	3.3
	14,400	1,800	8	19/20	2(4), 43(15)	16	6,853	5,388	7,492	
	14,300	1,586	9	5/20	20(4), 264(1)	30	8,778	8,066	9,553	
	13,900	1,986	7	4/20	21(2), 96(2)	50	12,591	12,155	13,042	
	6,200	886	7	7/20	<1(3), 24(2), 720(2)	84	24,946	20,552	30,278	
	5,540	1,385	4	0/20	–	99	62,350	40,619	95,704	
All rodents						1	384	0.6	251,830	1.0
						16	8,362	1,266	55,248	
						30	24,798	16,115	38,160	
						50	83,380	6,125	431,143	
						84	831,394	5,243	131,832,220	
						99	18,083,876	1,128	289,951,250,000	
All nonrodents						1	1,605	1,505	1,712	2.0
						16	7,635	7,418	7,859	
						30	13,237	13,015	13,462	
						50	24,462	24,277	24,648	
						84	78,332	76,222	80,502	
						99	–	–	–	
All species combined						1	176	1.8	17,512	0.97
						16	4,144	760	22,595	
						30	12,634	6,266	25,476	
						50	43,808	24,549	78,178	
						84	463,066	31,124	6,889,632	
						99	10,890,876	39,867	2,975,150,600	

* Number in parenthesis indicates number of mortalities at the given times; otherwise a single mortality occurred.

Table XV. Acute Inhalation Toxicity of DM Disseminated From No. 113 Federal Laboratories Spodeheat Munitions and a Bliss Statistical Analysis of the Mortality Responses

(30-Day observation; experiments performed in 1965)

Species	Ct	Concn	Exposure time	Mortality fraction	Times to death	Bliss statistical analysis				
						P	ED(P)	Lower limit	Upper limit	Slope
	mg min/cu m	mg/cu m	min		hr			mg min/cu m		
Monkey	29,000	3,222	9	6/6	3(2), * 5(2), 24, 28	1	8,131	1,097	60,252	5.2
	26,270	3,753	7	1/6	17	16	14,678	6,838	31,509	
	18,200	3,033	6	3/6	17(2), 332	30	18,080	12,460	26,235	
	14,600	2,433	6	1/6	42	50	22,814	16,297	31,936	
	10,400	2,600	4	0/6	—	84	35,459	10,730	117,182	
						99	64,007	5,539	739,593	
Dog	51,600	3,686	14	6/6	<1, 17(4), 41	1	9,699	2,881	32,659	5.0
	42,160	4,216	10	5/6	18(3), 96, 163	16	17,952	9,763	33,009	
	29,000	3,222	9	2/6	48, 70	30	23,309	14,686	33,889	
	26,270	3,753	7	2/6	48, 72	50	28,428	21,623	37,376	
	14,240	3,560	4	1/6	304	84	45,019	27,878	72,708	
						99	83,322	28,597	242,771	
Goat	97,800	3,622	27	6/6	<1(4), 16(2)	1	1,072	69	16,671	2.2
	77,500	3,875	20	6/6	16(5), 138	16	4,216	867	20,497	
	60,000	5,000	12	5/6	22, 240(2), 456, 648	30	6,837	2,084	22,433	
	50,550	3,611	14	6/6	17(2), 96, 144, 164, 264	50	11,723	5,335	25,763	
	36,135	4,015	9	5/6	68(2), 600, 624(2)	84	32,595	18,155	58,520	
	22,400	3,200	7	4/6	18, 316, 456, 720	99	128,200	26,528	619,545	
	13,140	3,285	4	4/6	18, 360, 472, 552					
	8,500	2,125	4	2/6	600, 672					
Swine	60,000	5,000	12	6/6	22(6)	1	20,874	7,405	58,837	9.9
	43,600	4,360	10	4/6	9(2), 22(2)	16	28,467	16,713	48,487	
	36,135	4,015	9	4/6	24(3), 672	30	31,761	22,016	45,821	
	22,400	3,200	7	0/6	—	50	35,888	28,856	44,637	
	13,140	3,285	4	0/6	—	84	45,245	32,265	63,446	
						99	61,704	27,086	140,564	
Rabbit	97,800	3,622	27	12/12	16(12)	1	16,894	8,279	34,475	5.2
	77,500	3,875	20	12/12	17(12)	16	30,333	21,509	42,777	
	60,700	4,669	13	2/6	18(2)	30	37,294	29,534	47,092	
	51,600	3,686	14	2/6	17, 41	50	46,959	39,615	55,665	
	42,160	4,216	10	2/6	18, 96	84	72,698	51,964	101,704	
	34,600	4,325	8	1/6	216	99	130,527	64,541	263,975	
	29,600	3,222	9	4/6	2(2), 24(2)					
	26,270	3,753	7	0/6	—					
	25,725	3,675	7	0/6	—					
	14,600	2,433	6	0/6	—					
	14,240	3,560	4	0/6	—					
Guinea pig	77,500	3,875	20	39/40	1(19), 17(20)	1	9,361	4,325	20,261	4.6
	51,600	3,686	14	17/20	<1, 17(16)	16	18,195	12,624	26,225	
	50,550	3,611	14	20/20	>1(9), 17(11)	30	23,005	18,282	28,950	
	42,160	4,216	10	15/20	2(2), 18(13)	50	29,888	26,615	33,564	
	29,000	3,222	9	17/20	2(6), 4(6), 24(4), 597	84	49,096	36,633	65,816	
	26,270	3,753	7	0/20	—	99	95,432	47,872	191,439	
	25,725	3,675	7	0/20	—					
	14,600	2,433	6	1/20	18					
	14,240	3,560	4	6/20	18, 42, 402(2), 448, 449					
	8,500	2,125	4	0/20	—					
Rat	97,800	3,622	27	39/40	16(38), 32(1)	1	11,528	3,768	35,578	3.8
	77,500	3,875	20	35/40	17(33), 33(2)	16	26,202	15,778	43,514	
	51,600	3,686	14	10/20	17(7), 696(3)	30	34,958	25,935	47,120	
	50,550	3,611	14	2/20	17(2)	50	48,217	42,489	54,718	
	42,160	4,216	10	1/20	19	84	88,730	56,188	140,117	
	29,000	3,222	9	12/20	24(12)	99	200,801	68,819	585,899	
	26,270	3,753	7	0/20	—					
	14,600	2,433	6	3/20	18(2), 42					
	14,240	3,560	4	0/20	—					

* Number in parenthesis indicates number of mortalities at the given time; otherwise, a single mortality occurred.

Table XV. (contd)

Species	Ct	Concn	Exposure time	Mortality fraction	Times to death	Bliss statistical analysis				
						P	ED(P)	Lower limit	Upper limit	Slope
	mg min/cu m	mg/cu m	min		hr		mg min/cu m			
All rodents						1	8,665	4,030	18,628	3.6
						16	20,192	14,243	28,627	
						30	27,220	22,095	33,532	
						50	37,980	34,593	41,699	
						84	71,439	52,477	90,401	
All nonrodents						1	4,988	2,172	11,453	3.0
						16	13,948	9,411	20,673	
						30	30,053	15,576	25,816	
						50	30,063	25,848	34,965	
						84	64,794	45,804	91,656	
						99	181,199	82,966	395,738	
All species combined						1	5,823	111	306,223	3.0
						16	16,714	2,792	93,705	
						30	23,197	8,711	61,770	
						50	34,685	30,245	39,773	
						84	74,374	15,886	348,206	
						99	206,579	4,862	8,776,949	

a. The Influence of Concentration, Time, and Ct on Deaths Caused by Acute Exposures to DM.

Lethality of DM in animals appears to be related to Ct rather than to concentration or time, individually. The relationships between Ct and mortality can be seen through all the individual experiments in tables X, XII, XIV, and XV. The most striking evidence of the greater importance of Ct is the data for monkeys exposed to pure DM disseminated from 10% acetone solutions and for DM thermally generated from the M6A1 or No. 113 grenades. The LCt50's for monkeys are 17,837 (15,351 to 20,725), 19,569 (14,193 to 26,980), and 22,814 (16,297 to 31,936) mg min/cu m, respectively, for the three methods of aerosol generation. The value for pure DM was obtained at concentrations of 209 to 297 mg/cu m and exposure times of 20 to 135 min. The LCt50's for the M6A1 and No. 113 grenades were obtained at concentrations of 1,800 to 2,808 and 2,600 to 3,222 mg/cu m, respectively, and exposure times of 7 to 13 and 4 to 9 min, respectively.

In rats, guinea pigs, rabbits, dogs, and monkeys, the LCt50's for pure agent dispersion (long exposure times, low agent concentration) were greater than for the M6A1 and the No. 113 grenade dispersions (short exposure times, high agent concentration). In swine and goats the reverse was true.

b. Times to Death.

Times to death for the various animals in the different experiments are shown in tables X, XII, XIV, and XV. Table XVI summarizes the mortalities produced in the seven animal species exposed to DM acetone sprays and DM from the M6A1 and the No. 113 grenades.

In all species and at most Ct levels, some deaths occurred in 1 day or less. With airborne sprays, about 60% of the deaths occurred during the first 2 days and 80% in less than 1 wk. With the M6A1 munition, about 84% and an additional 9% (93%) of the deaths occurred in 2 and 7 days, respectively, but with the No. 113 munition, 89% and 92% of the deaths occurred in the same time period. Only a few deaths were delayed beyond 2 wk with either of the three systems.

c. Summary of Animal Mortality Following Acute Exposures to DM.

The human population to which we must project our toxicity estimates is highly heterogeneous. Various persons come from various genetic strains. Genetic responses to the effects of drugs are variant.

Table XVI. Summary of Times to Death Following Inhalation of DM in Rats, Guinea Pigs, Rabbits, Dogs, Monkeys, Goats, and Swine

(Experiments performed in 1965)

Time to death	Acetone spray			M6A1 munition			No. 113 munition		
	No. of deaths	Cumulative No. of deaths	Cumulative % of deaths	No. of deaths	Cumulative No. of deaths	Cumulative % of deaths	No. of deaths	Cumulative No. of deaths	Cumulative % of deaths
day									
<1	57	57	27	50	50	26	281	281	85
1	52	109	51	77	127	65	9	290	88
2	32	141	66	37	164	84	5	295	89
3	12	153	71	9	173	89	1	296	89
4	7	160	74	4	177	91	3	299	90
5	14	174	81	–	177	91	2	301	91
6	4	178	83	2	179	92	2	303	92
7	2	180	84	1	180	93	–	303	92
8	8	188	87	–	180	93	–	303	92
9	3	191	89	–	180	93	1	304	92
10	7	198	92	5	185	94	2	306	92
11	3	201	93	1	186	95	1	307	93
12	3	204	95	2	188	96	1	308	93
13	2	206	96	–	188	96	2	310	94
14	4	210	97	–	188	96	–	310	94
15	1	211	98	1	189	97	1	311	94
16	–	211	98	–	189	97	2	313	94
18	–	211	98	2	191	98	2	315	95
19	–	211	98	2	193	99	3	318	96
22	2	213	99	–	193	99	–	318	96
23	2	215	100	–	193	99	1	319	96
24	–	215	100	–	193	99	1	320	97
25	–	215	100	–	193	99	2	322	97
26	–	215	100	–	193	99	2	324	98
27	–	215	100	–	193	99	1	325	98
28	–	215	100	–	193	99	2	327	99
29	–	215	100	–	193	99	3	330	99
30	–	215	100	2	195	100	1	331	100

The human population contains persons whose living habits, eating habits, histories of diseases, drug-taking, and environmental exposures to gasolines, metal fumes, dust, pollows, etc., are completely different. The conditions of the various individuals before, during, and after exposure will be very different. To project estimates to such a heterogeneous human population, the animal population exposed to DM should be large and heterogeneous. (These factors are accounted for, to the greatest degree, by using the data for all species of animals combined.)

Single experiments on any species have examples of high or low LCt50's and high or low slopes. Whenever data are summated to include many animals and wider variability (animals, and other conditions), the LCt50's for DM average 10,000 to 20,000 mg min/cu m, and the slope of the regression line flattens to 1.0 to 2.0. This wide variability holds for experiments conducted during 1918 to 1964 (total of 868 animals). It is also true for experiments conducted with pure DM during 1965 (total of 407 animals), 1965 studies with the M6A1 and No. 113 munitions (total of 1,129 animals), and the summation of all the studies (2,404 animals).

This is to be expected, since any individual experiment involves a segment of the overall population. This segment is likely to include animals that are relatively homogeneous, especially the rodents, which, in all probability, would be littermates. It applies largely to rabbits, pigs, goats, and, to some degree, monkeys. The dogs are usually mongrels, but for any given experiment, the animals are in a group that is housed and handled together for periods usually greater than 1 mo. Thus, handling, environmental conditions, experimental conditions, and many other variables are relatively constant for all animals in a given experiment. The animals and the conditions may be different from one experiment to another. The variables that could influence toxicity are difficult to detect; therefore, reactions to drugs or chemicals may be much more widespread than is realized. An outstanding example is the metabolic action of tranylcypromine. Persons taking this drug become severely poisoned by normally innocuous foodstuffs, such as cheese.[19,20] Another example is mercaptopurine, which inhibits the ability to produce antibodies in response to antigens.[21]

Exposure to an agent may sensitize the animals to a disinfectant used to scrub the floor, to the absorbent substance spread on the bottoms of the cages, or to minor infections or colds among the laboratory and animal-colony personnel. The lettuce fed to the guinea pigs may come from different parts of the US in different seasons of the year. It is therefore possible that insecticides used on the food could influence the toxicity of the experimental agent. Such variables would be different from experiment to experiment.

5. Subacute Exposures.

Laboratory - Aerosol Branch; investigators, T. W. Ballard, G. F. Egan, J. T. Weimer, T. L. Hess, G. F. Sell, R. L. Farrand, J. S. Olson, and R. P. Merkey; 1964.

Two groups of eight monkeys, eight dogs, and 20 guinea pigs each were exposed for 10 consecutive days to aerosols of DM generated from the No. 113 grenade (table XVII).

The animals from group 1 were exposed to daily Ct's of DM ranging from 9,740 to 13,720 mg min/cu m (monkey LCt3 to LCt13, dog LCtl to LCt6, guinea pig LCtl.2 to LCt6). The average daily Ct was 11,609 mg min/cu m (monkey LCt7.0, dog LCt2.7, guinea pig LCt3). The 10-day cumulative Ct was 116,090 mg min/cu m (monkey LCt99.99, dog LCt99.90, guinea pig LCt99.7).

Two monkeys died on the 11th day, one on the 12th day, one on the 13th day, and one on the 24th day, for a total of five out of eight dead. Only one of the eight dogs died (16th day). One guinea pig died on the ninth day, two on the 12th day, and three on the 20th day, for a total of three out of 20 dead. Monkey mortality was greater than would be expected from any of the daily exposures alone, but less than would be expected from the 10-day cumulative Ct. Dog and guinea pig mortalities were not greater than would be expected from any of the daily exposures, but were far less than would be expected from the 10-day cumulative Ct.

The animals from group 2 were exposed to daily Ct's of DM ranging from 14,540 to 21,660 mg min/cu m (monkey LCt16 to LCt46; dog LCt8 to LCt28, guinea pig LCt8 to LCt26). The average daily Ct was 17,302 mg min/cu m (monkey LCt28, dog LCt15, guinea pig LCt15). The 10-day cumulative Ct was 173,020 mg min/cu m (monkey LCt $<$ 99.9, dog LCt $>$ 99.9, guinea pig LCt99.99).

One monkey died on the second day following the exposure. All eight monkeys were dead by the 17th day. One guinea pig died after the first exposure. Additional guinea pigs died on the eighth day, and by the 12th day, 18 had died. The resultant 30-day mortality fraction in guinea pigs was 18/20. One dog died on the second day and one on the fifth day. The resultant 30-day mortality fraction in dogs was 2/8.

Table XVII. Subacute Inhalation Toxicity of DM Disseminated From No. 113 Federal Laboratory Munition in Guinea Pigs, Dogs, and Monkeys

(Exposures daily for 10 days)

Day	Group I daily Ct	Cumulative Ct	Cumulative deaths			Daily Ct	Cumulative Ct	Cumulative deaths		
			Guinea pig	Dog	Monkey			Guinea pig	Dog	Monkey
	mg min/cu m					mg min/cu m				
1	9,740	–	0/20	0/8	0/8	16,620	–	1/20	0/8	0/8
2	12,020	21,760	–	–	–	16,020	32,640	–	1/8	1/8
3	11,060	32,820	–	–	–	17,560	50,200	–	–	2/8
4	11,000	43,820	–	–	–	15,920	66,120	–	–	–
5	12,920	56,740	–	–	–	16,920	83,040	–	2/8	–
6	11,620	68,360	–	–	–	17,360	100,400	–	–	3/8
7	11,750	80,110	–	–	–	14,540	114,940	–	–	–
8	9,940	90,050	–	–	–	19,020	133,960	3/20	–	–
9	13,720	103,770	1/20	–	–	21,660	155,620	4/20	–	–
10	12,320	116,090	2/20	0/8	0/8	17,400	173,020	4/20	2/8	3/8
11			2/20	–	2/8			12/20	–	4/8
12			–	–	3/8			18/20	–	6/8
13			–	–	4/8			–	–	–
16			–	1/8	–			–	–	–
17			–	–	–			–	–	8/8
20			3/20	–	–			–	–	–
24			–	–	5/8			–	–	–
30			3/20	1/8	5/8			18/20	2/8	8/8

Note: The acute lethal Ct's for DM disseminated from the No. 113 grenade are:

Monkey		Dog		Guinea pig	
P	Ct mg min/cu m	P	Ct mg min/cu m	P	Ct mg min/cu m
1	8,131	1	9,699	1	9,361
16	14,678	16	17,952	16	18,195
30	18,080	30	23,309	30	23,005
50	22,814	50	28,428	50	29,888
84	35,459	84	45,019	84	45,096
99	64,007	99	83,322	99	95,432

The monkey and guinea pig 30-day mortality ratios were greater than would be expected from any of the daily exposures alone but would be expected from the 10-day cumulative Ct. The dog mortality ratios would be expected from any of the daily exposures alone, but were far less than would be expected from the 10-day cumulative Ct.

There was little indication of cumulative toxicity due to the repeated exposures. The data are shown in table XVII.

B. Influence of Solvents.

There is an indication that DM dispersed in pure form is more toxic than DM dispersed from the M6A1 grenade or the No. 113 grenade. Since acetone was often used in the dispersion of pure DM, the solvent may have increased the inhalation toxicity. Twenty-five rats and guinea pigs were exposed to DM from the No. 113 grenade. The airborne concentration, exposure time, and Ct were 2,531 mg/cu m, 7 min, and 17,717 mg min/cu m, respectively. Acetone was sprayed into the chamber during the entire exposure.

The mortality fraction from exposure to DM (No. 113 grenade) plus the acetone was 2/25 for the rats and 2/25 for the guinea pigs. These results are similar to those produced by DM without acetone at a Ct of 14,600 mg min/cu m: 3/20 for the rats and 1/20 for the guinea pigs.

The mortality fraction from DM (acetone spray) at a Ct of 16,700 mg min/cu m was 1/20 for the rats and 16/20 for the guinea pigs. The data indicate that DM dispersed from the munitions is less toxic than DM dispersed in the pure form and that this toxicity is not increased by adding acetone.

C. Pathology.

1. Gross and Microscopic.

Pathological changes resulting from inhalation of DM have been reported by Ransom and Bogart[4] (dogs), Downing and Sternberger[22] (mice), Punte and coworkers[16] (mice, rats, and guinea pigs), Hazleton Laboratories[15] (dogs and monkeys), and Streett and Striker[23] (rats, guinea pigs, dogs, monkeys, goats, and swine).

Dogs dying from exposure to DM had hyperemia of the larynx and trachea, edema and congestion of the lung, and bronchopneumonia. Similar lesions were noted in mice, rats, monkeys, swine, and goats following inhalation of DM.[4, 15, 16, 24]

Fibrin clots were found in the hearts of some dogs that died after exposure to DM.

Liver damage was also reported in mice after inhalation of Ct's of 4,000 to 5,000 mg min/cu m. After 3 days, there were generalized icterus and areas of focal necrosis with and without hemorrhage in the liver. Cell infiltration was noted around the bile ducts.[22]

The following is quoted verbatim from Ransom and Bogart.[4]

> 1. In the dogs which died after being gassed at concentrations at and above 0.62 mg/liter there was evidence of acute and marked damage of the upper respiratory tract as follows:
>
> | | | |
> |---|---|---|
> | a. | Hyperemia larynx and trachea | 100% |
> | b. | Pseudo membranous tracheitis | 60% |
> | c. | Acute edema of lungs | 100% |
> | d. | Congestion of lungs | 100% |
> | e. | Bronchopneumonia | 30% |
>
> 2. In the four dogs dead after exposure to below 0.62 mgm/liter all of which had delayed deaths, there was:
>
> | | | |
> |---|---|---|
> | a. | Purulent conjunctivitis | 100% |
> | b. | Hyperemia larynx and trachea | 50% |
> | c. | Bronchopneumonia | 100% (1 animal) |
> | d. | Pseudo membranous tracheitis | 25% (1 animal) |
>
> 3. The following animals, killed for autopsy, showed practically nothing and had apparently entirely recovered from the exposure.

Dog	Concentration (Nominal Anal)		Days Following Exposure
	mg/cu m		
CL 675	1.02	1.01	12 days
CL 588	0.91	0.81	15 days
CL 655	0.80	0.60	12 days
CL 624	0.617	0.60	14 days
CS 741	0.17	0.14	9 days

The following was reported by Downing and Sternberger.[22]

> Summary - Mice exposed to an inhalation dose of Ct's 4,000 to 6,000 of DM dust showed generalized icterus in 3 days with liver damage indicated by focal areas of necrosis with and without hemorrhage, cell infiltration around bile channels, and parenchymal regeneration underway. A week after exposure, parenchymal repair was progressing, but irritation of biliary vessels had increased with cellular infiltration of thickened walls and metaplasia of the epithelial lining. Two weeks after exposure the parenchyma was essentially normal, but the changes noted in the biliary systems one week after exposure were still seen.

The following pathology was reported by Punte and coworkers.[11]

> Mice, rats, and guinea pigs sacrificed or dying after exposures to diphenylaminochloroarsine aerosol Ct's greater than 500 mg min/cu m revealed hyperemia of the trachea, pulmonary congestion, and edema and pneumonia. Animals exposed to Ct's below 500 mg min/cu m revealed no pathology.

The following is quoted from the Hazleton Laboratories Report.[15]

> As was the case with pharmacotoxic manifestations of exposure to the irritants, general gross pathological changes were similar for all species. The most commonly occurring abnormalities included: varying degrees of pulmonary congestion, an apparent splenic contraction, congestion of the tracheal mucosa, congestion of the liver which was also pale in color, petechial hemorrhage and diffuse congestion of the small intestine, severe hemorrhage of the colonic villi, and congestion of the kidneys. In addition to these grossly observable signs of general pathological modification, other abnormalities which seemed to be characteristic of the particular species or the specific mixture were observed.
>
> A distinctive observation made in dogs that had been exposed to pure DM was a white fibrin clot in both cardiac ventricles of 5 of the dogs which expired within 7 days after the exposure.

In addition, the entire intestinal tracts of several of the dogs which recovered after the exposure were severely congested. Other observations in the dogs included: mottled and hemorrhagic kidneys with poor cortico-medullary differentiation, multiple dark red longitudinal spots in the intestinal mucosa, and the gastric rugae were prominent and particularly congested in the fundus.

In the monkeys, liver involvement appeared to be more pronounced than in the case of the dogs. Also, diffuse hemorrhage of the gastric and intestinal mucosa was noted and the intestines were generally filled with mucus and a bile-like substance. The lungs were severely congested with a blood-tinged serous fluid and a thickened mucosal epithelium was common.

The following section (quoted verbatim) describes gross pathological findings seen after exposure to various dissemination systems.[23]

Pure DM.

The acute and chronic pathologic effects of DM aerosol on several different animal species were investigated.

Dogs, monkeys, goats, pigs, guinea pigs, and rats were exposed to various Ct's via the aerosol route. Both the acute and chronic pathologic changes in multiple organ systems were determined. This report is a preliminary report giving the pathologic changes seen grossly of all of the animals autopsied. Tissues from the same animals are currently being studied to determine the histopathologic effects. A report on a representative sample of these animals will follow at a later date.

Results - All organ systems were examined with the exception of the nervous system. The respiratory system was universally affected by the agent in all species and showed marked pathologic changes. In the early stages, where animals died acutely, pulmonary edema, pulmonary congestion, pulmonary hemorrhages, laryngeal edema, laryngeal congestion, and tracheitis predominated.

Given time, the ones which survived generally all developed pneumonia which was present at both the 14- and 30-day post exposure time periods. This was true in all of the goats (4/4) and pigs (3/3) and in all but one of the monkeys (6/7). Also, all but 2 of the dogs (4/6) developed a pneumonia. These 2 were at relatively low dosages (Ct 3000 and 9000) and a 30-day post exposure.

In the smaller laboratory animals, rats and guinea pigs, at the 30-day post exposure, only one guinea pig (1/5) showed any gross lesions.

The cardiovascular system was affected in one animal in each of 3 species (dog, goat, monkey) as evidenced by hemorrhages in the heart. These could have resulted terminally in the animals and not have been directly attributable to the agent.

In the dog, pathologic damages were also found in the gastrointestinal system, kidney, liver, and eye; however, nothing definite can be said as to their etiology and pathogenesis at this time. This is also true about the liver abscesses seen in one pig.

Summary - Dogs, monkeys, goats, swine, rats and guinea pigs were exposed to various Ct's of DM aerosol and the gross pathologic changes were observed. The respiratory system was the system universally affected. The most important pathologic changes noted were pulmonary edema, congestion, and hemorrhages, laryngeal congestion, tracheitis, and pneumonia.

The following is quoted verbatim from an informal pathology report presented to the Aerosol Branch in September 1965.

M6A1 DM Munition. The acute and 30 day pathologic effects of DM Munition in several different animals species were investigated.

Dogs, rhesus monkeys, swine, goats, rabbits, guinea pigs, and rats were exposed to aerosols of DM to various Cts by the inhalation route. This work was conducted by the Aerosol Branch, Toxicology Division, Dir of Med Res.

Representative numbers of the animals were autopsied at death, during early time periods, and a similar number were autopsied after 30 days of observation. This report considers only the gross pathologic changes. The histopathologic changes will be the subject of a future report.

Results - Five dogs were examined: four animals exposed to Cts of 21,200 to 29,500 died between 41 to 72 hours. All exhibited pulmonary edema, hemorrhage, congestion, as well as edema and congestion of the larynx. Tracheitis was present in all. Hemorrhagic gastroenteritis was present in one animal and chronic nephritis was present in one animal.

At 30 days, one dog exposed to a Ct of 29,500 had focal areas of pneumonia as well as chronic nephritis.

Monkey - Nine animals were examined. The time to death of the early animals varied from 18 to 48 hours. In most of these animals, there was pulmonary edema and hemorrhage with congestion. Edema and congestion of the larynx was also universally present. At the higher Ct's tracheitis was present. Conjunctival hemorrhage was present in one of the animals living to 48 hours. There was, as well in this group one animal who had epistaxis. In the animal surviving to 30 days exposed to a Ct of 24,200 pleural adhesions were noted.

Goat - Six animals were examined. Of these, five were animals during the acute period varying from 3 to 18 hours to time of death. The Ct's varied from 22,150 to 33,600. In these early animals, pulmonary edema, hemorrhage and congestion was present. In most of the animals there were, in addition, hemorrhages in the heart, edema and congestion of the larynx and, most strikingly, a pseudomembranous tracheitis in those animals living a sufficient length of time. One animal had liver abscesses and another was pregnant.

That animal surviving to 30 days exposed to a Ct of 12,200 had no gross lesions.

Pigs - Eight animals in all were studied. Of these, 7 died during the acute period varying from 18 to 48 hours. The Cts varied from 17,800 to 62,700. The severity of the

lesions did not appear to vary directly with the dose. In general, pulmonary edema hemorrhage and congestion was present in all and in the higher doses edema, hemorrhage and congestion of the larynx was present. Tracheitis was almost universal. Sub-endocardial hemorrhages were present in the higher dosages. Pneumonia and atelectasis was present in an occasional animal. Hepatic, parasitic infestation were present in some of the animals also.

At thirty days, one animal exposed to a Ct of 39,000 had pneumonia.

Guinea Pig - Four animals were observed during the acute period. The Ct's varied from 14,400 to 21,800. The times to death varied from 18 to 24 hours. Pulmonary edema, hemorrhage and congestion were the only lesions seen grossly.

Rabbits - Four animals were examined. Of these, three died during the period from 18 to 24 hours. The Ct's ranged from 32,700 to 39,000. One animal had pulmonary edema and the rest had pulmonary hemorrhages and congestion. Tracheitis was present in all as was edema of the soft palate. Hepatic coccidiosis was present in one animal. One animal submitted for autopsy at 30 days who was exposed to a Ct of 39,000 had no gross lesions.

Rat - At 30 days, one rat exposed to a Ct of 80,000 was submitted for autospy. Chronic murine pneumonia was present in this animal. A second rat with the similar post exposure and Ct had no gross lesions.

Summary - Dogs, monkeys, pigs, goats, guinea pigs, rabbits, and rats were exposed to various Ct's of DM munition and gross pathologic changes were observed. The upper respiratory system and lungs proper were almost universally involved during the early time periods. The severity of involvement varied somewhat between species. However, pulmonary edema and congestion was almost universal. Tracheitis and edema of the larynx were common findings in those animals surviving long enough. By 30 days, many animals showed no significant residue.

2. Blood Chemistry.

Laboratory No. 1 - Hazleton Laboratories, 1963.[15]

The hematological findings in dogs after exposure to the various levels of pure DM revealed no significant changes.

In monkeys, one animal at a Ct of 1610 (monkey No. 258) and both animals at a Ct of 14,400 exhibited an increase in the number of neutrophils and decreased lymphocyte counts 15 days post exposure and animals at a Ct of 19,500 (monkey No. 22W) exhibited these changes at 30 days post-exposure. Both animals at a Ct of 19,500 exhibited marked elevations in leukocyte counts 30 days post exposure and slight erythopenia 15 days post-exposure.

Serum transaminase and alkaline phosphatase activities were markedly increased at both 15 and 30 days post-exposure in one dog (Dog No. 5782) exposed at a Ct of 14,400, while only serum transaminase activity was increased 15 days post-exposure in the other at this level (Dog No. 5846).

In the monkeys exposed to pure DM, the only abnormal biochemical values were found in serum transaminase and alkaline phosphatase activities. One monkey (Monkey No. 258) exhibited an increase in serum transaminase activity 15 days post-exposure at a Ct of 1,610, while one animal (Monkey No. 66W) exposed to a Ct of 14,400 exhibited a tendency toward slightly decreased serum transaminase activity 15 and 30 days post-exposure, as well as decreased alkaline phosphatase activity at these intervals.

Tables XVIII and XIX present the hematological findings in dogs and monkeys, respectively, prior to and after exposure.

Laboratory No. 2 - Aerosol Branch; investigators, R. L. Farrand, T. L. Hess, S. G. Ryan, J. Vondruska, J. Burns, G. F. Sell, G. Anderson, W. M. Lawson, and G. F. Egan; 1965.

Periodic blood samples were taken from monkeys, dogs, goats, and swine during the 30-day postexposure observation period. In the acute studies, with pure DM spray and DM disseminated from the M6A1 and

No. 113 grenades, blood samples were taken 1, 7, 14, and 30 days post-exposure. In the subacute studies with the No. 113 grenades, samples were taken 3, 6, 9, 15, and 30 days postexposure.

Two samples of blood were required in each case: 8 ml was allowed to clot and the serum was collected, and 4 ml of whole blood was placed in an oxalated tube for hematological studies and determinations where whole blood or plasma was required. All blood was refrigerated when determinations were not being made. An attempt was made to complete all determinations related to enzyme or enzymatic processes within 3 days after the sample was taken.

The following methods or instrumentation, or both, were employed to make these determinations.

Determination	Methods and instruments
Red and white blood cells	Coulter counter
Hematocrit	Microhematocrit method
Prothrombin time	Mechrolb Inc.
Potassium	Flame photometer
SGOT and SGPT	Method of Reitman and Frankel modified
Creatinine and BUN	Technicon AutoAnalyzer
LDH	Method of Cabaub and Wrobluski
Total serum protein	Refractive index

Results of these tests on dogs and monkeys are given in tables XVIII and XIX, respectively.

a. Summary of Determinations.

(1) RBC and Hematocrit.

For the most part, the erythrocyte count did not change after animals were exposed to DM dispersed from either the M6A1 on No. 113 thermal grenades. Hematocrit values for the monkey decreased significantly after acute exposure of the animals to the M6A1 munition and after subacute exposure to the No. 113 grenade. Hematocrit values for the dog also decreased after subacute exposure to this grenade. Hematocrit values for goats and swine decreased slightly after acute exposure of the animals to this grenade.

Table XVIII. Hematological and Biochemical Values for Mongrel Dogs Receiving Specified Doses of Pure DM by Inhalation

Dog No.	Dosage level	Time interval	Cell volume	Hemoglobin	RBC per cu mm ($\times 10^6$)	WBC per cu mm	Differential							Sed rate	Prothrombin time	B
							Myel + meta	Juv + band	Seg	Lymph	Mono	Eosin	Baso			
	Ct	days	%	gm/100 ml			%							mm/hr	sec	m
7005 (F)	1,610	Initial	40.0	13.6	5.50	24,600	0	0	70	27	2	1	0	0.5	8.2	1
		15	39.0	13.4	5.50	9,100	0	0	65	35	0	0	0	2.5	8.0	1
		30	45.0	14.8	6.48	17,500	0	0	71	28	0	1	0	0.5	8.9	2
7006 (F)	1,610	Initial	42.5	14.6	5.87	24,400	0	0	51	47	1	1	0	1.5	8.9	1
		15	44.0	15.4	6.08	15,000	0	0	71	29	0	0	0	0.5	9.5	1
		30	45.0	15.7	6.44	14,000	0	0	62	35	1	2	0	0.5	8.8	1
5782 (M)	14,400	Initial	47.5	16.0	6.68	11,900	0	1	69	24	1	5	0	2.0	7.5	1
		Initial	43.0	14.3	6.04	14,100	0	0	72	22	2	4	0	6.0	7.8	1
		15	39.5	13.1	5.55	20,400	0	0	88	9	1	1	1	1.0	7.3	1
		30	38.0	13.4	5.56	15,300	0	0	86	7	2	5	0	23.0	8.0	1
5846 (F)	14,400	Initial	37.5	12.1	5.70	17,800	0	7	84	9	0	0	0	52.0	7.0	
		Initial	37.5	14.9	5.80	18,000	0	2	57	37	3	1	0	22.0	7.1	1
		15	39.5	12.6	5.42	23,300	0	0	87	11	0	1	1	1.0	7.0	1
		30	43.0	14.2	5.21	14,700	0	1	79	14	2	4	0	1.0	7.3	1

Table XIX. Hematological and Biochemical Values for Rhesus Monkeys (Macaca mulatta) That Received Specified Doses of Pure DM by Inhalation

Monkey No.	Dosage level	Time interval	Cell volume	Hemoglobin	RBC per cu mm ($\times 10^6$)	WBC per cu mm	Differential							Sed rate	Prothrombin time	Bromsulfalein
							Myel + meta	Juv + band	Seg	Lymph	Mono	Eosin	Baso			
	Ct	days	%	gm/100 ml			%							mm/hr	sec	% 30 min
258 (M)	1,610	Initial	44.0	13.1	6.42	10,800	0	0	32	65	1	2	0	0.5	13.4	0
		15	42.0	12.8	5.93	12,500	0	0	63	36	0	1	0	1.0	15.0	0
		30	40.5	12.8	6.28	8,300	0	0	30	68	1	1	0	0.5	15.3	0
263W (M)	1,610	Initial	39.0	14.2	5.58	12,900	0	0	41	57	0	2	0	1.0	13.4	0
		15	40.0	13.2	6.18	12,200	0	0	37	62	0	1	0	0.5	15.3	0
		30	43.0	13.6	6.30	12,700	0	0	37	62	0	1	0	0.5	13.9	0
64W (M)	14,400	Initial	41.0	14.2	5.55	12,600	0	0	26	72	1	1	0	0.5	14.9	0
		Initial	39.5	12.5	5.41	13,600	0	1	43	54	0	2	0	1.0	13.8	0
		15	39.5	11.8	5.42	18,600	0	0	65	31	0	0	4	1.0	12.8	0
		30	37.0	11.7	5.14	10,100	0	0	38	59	0	3	0	1.0	13.7	0
66W (M)	14,400	Initial	40.5	14.0	5.91	8,000	0	0	42	58	0	0	0	0.5	14.0	0
		Initial	38.5	12.6	5.47	7,500	0	0	48	50	2	0	0	1.0	13.9	0
		15	33.5	11.3	4.77	10,700	0	9	60	36	0	3	1	2.0	13.0	0
		30	34.5	11.1	5.07	7,300	0	0	48	44	1	6	1	1.0	13.7	0
22W (M)	19,500	Initial	43.0	13.3	5.80	8,400	0	0	39	59	0	2	0	0.5	14.0	0
		Initial	38.0	12.5	5.42	9,800	0	0	34	59	0	7	0	1.2	15.2	0
		15	37.5	11.7	4.82	12,600	0	0	27	71	2	0	0	2.0	13.5	0
		30	39.0	12.1	5.68	29,200	0	2	59	38	1	0	0	0.1	13.4	0
30W (M)	19,500	Initial	34.0	11.5	5.14	10,800	0	0	58	38	0	4	0	0.5	13.9	0
		Initial	33.5	11.3	4.69	11,300	0	0	51	46	0	3	0	1.5	15.1	0
		15	36.5	12.0	5.07	18,800	0	0	57	38	3	2	0	7.5	18.7	0
		30	39.0	12.1	5.68	29,200	0	2	59	38	1	0	0	0.1	11.6	0

Table XVIII. Hematological and Biochemical Values for Mongrel Dogs Receiving Specified Doses of Pure DM by Inhalation

Differential							Sed rate	Prothrombin time	BUN	Sugar	Sodium	Potassium	Chlorides	Serum transaminase	Alkaline phosphatase
Myel + meta	Juv + band	Seg	Lymph	Mono	Eosin	Baso									
%							mm/hr	sec	mg %	mg/100 ml	meq/l			units	
0	0	70	27	2	1	0	0.5	8.2	12.0	70	154	5.4	108	4	0.9
0	0	65	35	0	0	0	2.5	8.0	13.8	111	151	5.4	107	11	1.6
0	0	71	28	0	1	0	0.5	8.9	22.0	118	151	5.2	106	1	2.0
0	0	51	47	1	1	0	1.5	8.9	12.0	88	153	5.2	108	7	2.4
0	0	71	29	0	0	0	0.5	9.5	12.5	89	150	5.3	103	12	2.2
0	0	62	35	1	2	0	0.5	8.8	16.0	96	148	5.1	107	1	2.0
0	1	69	24	1	5	0	2.0	7.5	15.5	91	168	7.3	119	14	0.8
0	0	72	22	2	4	0	6.0	7.6	10.5	102	151	6.5	111	16	0.8
0	0	88	9	1	1	1	1.0	7.3	14.5	102	158	4.8	112	70	7.2
0	0	86	7	2	5	0	23.0	8.0	12.0	107	147	5.4	107	196	20.3
0	7	84	9	0	0	0	52.0	7.0	9.5	67	143	4.8	112	5	1.1
0	2	57	37	3	1	0	22.0	7.1	12.0	75	141	4.9	117	4	1.1
0	0	87	11	0	1	1	1.0	7.0	15.0	85	147	5.7	110	84	1.9
0	1	79	14	2	4	0	1.0	7.3	12.5	108	155	5.5	110	9	1.1

Table XIX. Hematological and Biochemical Values for Rhesus Monkeys (Macaca mulatta) That Received Specified Doses of Pure DM by Inhalation

Differential							Sed rate	Prothrombin time	Bromsulfalein	BUN	Sugar	Sodium	Potassium	Chlorides	Serum transaminase	Alkaline phosphatase
Myel + meta	Juv + band	Seg	Lymph	Mono	Eosin	Baso										
%							mm/hr	sec	% 30 min	mg %	mg/100 ml	meq/l			units	
0	0	32	65	1	2	0	0.5	13.4	0	23.0	66	154	5.2	104	11	10.0
0	0	63	36	0	1	0	1.0	15.0	0	21.3	80	153	5.1	107	43	9.8
0	0	30	68	1	1	0	0.5	15.3	0	18.5	80	156	5.2	108	2	8.5
0	0	41	57	0	2	0	1.0	13.4	0	22.0	64	150	5.4	106	14	9.1
0	0	37	62	0	1	0	0.5	15.3	0	23.3	80	155	5.4	108	21	8.0
0	0	37	62	0	1	0	0.5	13.9	0	18.0	94	149	4.8	103	3	9.0
0	0	26	72	1	1	0	0.5	14.9	0	15.5	71	157	5.8	108	18	10.8
0	1	43	54	0	2	0	1.0	13.8	0	13.5	82	151	4.8	106	18	13.0
0	0	65	31	0	0	4	1.0	12.8	0	10.5	83	145	6.5	105	14	8.0
0	0	38	59	0	3	0	1.0	13.7	0	13.0	101	147	4.0	105	15	9.6
0	0	42	58	0	0	0	0.5	14.0	0	17.5	84	162	6.2	106	22	11.0
0	0	48	50	2	0	0	1.0	13.9	0	15.0	78	153	5.4	108	24	11.4
0	0	60	36	0	3	1	2.0	13.0	0	14.5	88	149	5.7	106	10	3.8
0	0	48	44	1	6	1	1.0	13.7	0	17.0	74	152	4.9	103	16	6.6
0	0	39	59	0	2	0	0.5	14.0	0	17.0	78	155	4.9	108	23	7.6
0	0	34	59	0	7	0	1.2	15.2	0	16.0	76	153	5.1	111	13	6.4
0	0	27	71	2	0	0	2.0	13.5	0	20.0	77	148	5.6	104	11	6.6
0	2	59	38	1	0	0	0.1	13.4	0	16.0	68	165	5.6	105	13	6.2
0	0	58	38	0	4	0	0.5	13.9	0	27.0	80	150	6.1	105	18	4.2
0	0	51	46	0	3	0	1.5	15.1	0	23.0	74	158	6.1	111	12	4.6
0	0	57	38	3	2	0	7.5	18.7	0	19.5	82	146	7.3	104	12	5.0
0	2	59	38	1	0	0	0.1	11.6	0	18.0	97	165	5.6	105	13	6.2

2

(2) WBC.

In general, the WBC count increased on the first day in many of the animals. The dogs and swine showed the least change. The count for the monkey generally increased after acute exposure to DM disseminated as a spray and from the M6A1 grenade and after subacute exposure to agent disseminated by the No. 113 grenade. The goat WBC count decreased after acute exposure to DM disseminated as a spray or from the No. 113 grenade.

(3) Lactic Dehydrogenase (LDH).

Goats and swine showed no change in LDH activity following exposures to DM by all methods of dispersion. The monkey LDH activity increased following exposure to DM disseminated as a spray and by the No. 113 grenade. The dog had a decrease in LDH activity following acute and subacute exposures to DM from the No. 113 grenade.

(4) Prothrombin Time.

Prothrombin time following exposure to the No. 113 grenade was measured. All dogs, goats, and swine had significant decreases in this value; however, no appreciable change was apparent in the monkey.

(5) Total Serum Protein.

No significant changes were noted in this measurement.

(6) Potassium.

The only change noted in potassium values was a slight decrease in the dog's value following subacute exposures to DM by the No. 113 grenade.

(7) Serum Glutamic Pyruvic Transaminase (SGPT) and Serum Glutamic Oxaloacetic Transaminase (SGOT).

An increase in SGPT and SGOT activities occurred only after exposures to the No. 113 grenades. The goat SGOT activity increased following an acute exposure. The dog and monkey had increases in both values after subacute exposures. The dog transaminase activities increased after both doses

(table XVIII), and the monkey transaminase activities increased only at the higher Ct value (173,020 mg min/cu m). These results tend to indicate a possible liver involvement.

(8) Blood Urea Nitrogen (BUN) and Creatinine.

The only change noted in BUN occurred in the monkey. Following an acute exposure to DM disseminated by the No. 113 grenade, BUN values decreased somewhat; they increased following subacute exposures. Creatinine values did not show any appreciable changes in any of the species or studies.

(9) Alkaline Phosphatase.

Alkaline phosphatase determinations were reported for the swine and goat after exposure to the DM spray and the M6A1 grenade. The alkaline phosphatase activity occasionally showed a statistically significant decrease; however, from the pathological standpoint, there were no significant changes.

b. Conclusion.

The major changes noted in the clinical biochemical studies are evident in the WBC count and transaminase activity. The initial increase in WBC tends to indicate a response to stress due to the highly irritating properties of the agent. The transaminase response may possibly be due to liver involvement.

Individual changes in some of these parameters were much more dramatic and showed evidence of pulmonary hepatic, and in a few instances, renal involvement. However, this was not confirmed by necropsy.

2. Cause of Death in Animals That Have Inhaled DM.

The cause of death is not certain; the probable primary cause, especially for deaths occurring during the first 3 to 4 days following exposure, is lung damage. Animals and men dying after exposure to DM exhibit extensive lesions of the lungs and the respiratory tract. In anesthetized dogs severely poisoned by inhalation of DM, the blood oxygenation is markedly lowered, despite increased rate and depth of breathing. None of the measured effects on blood elements or chemical constituents, liver functions, kidney function, blood pressure, heart rate, etc., could adequately account for most of the deaths observed. Although the primary cause of death is probably lung damage, secondary factors, as yet not characterized, cannot be disregarded.

IV. TOXICITY ESTIMATES FOR MAN.

A. Estimated LCt50 for Man.

An estimate for the toxicity of inhaled DM in man was established at CRDL in March 1959.[25] This estimate used toxicity data on mice and guinea pigs reported in CRDL Technical Memorandum 24-18[10] and data on dogs reported in EACD 145.[9] The LCt50's differed greatly. From these values, there was no way to ascertain the lethality in man or to relate the toxicity in man with that in any of the animal species studied. All of the toxicity data were combined, and a composite lethality dose-response regression line for mammals, including man, was established. From this curve, the LCt50 for a single exposure is 14,000 mg min/cu m.

More recently, toxicity data in animals indicate that gross variability in the lethal response to inhaled DM is to be expected. Marked variability was noted in different tests using a single species of animal or in the same test using heterogeneous groups (different species) of animals. Thus, great variability in lethal response is to be expected in heterogeneous populations of people exposed under highly variable conditions to DM in various mixtures and at varying Ct levels. Consequently, the combined inhalation data for DM in all species of animals offer the best estimate for the expected lethal response in a heterogeneous population subjected to highly variable conditions before, during, and after exposure to the irritants. The Bliss statistical analyses of the pertinent data are shown in table XX.

In July 1966, the previous human LCt50 estimate of 14,000 mg min/cu m for inhaled DM dispersed by laboratory methods was reviewed by the Research Laboratories Human Estimate Committee, and the value of 11,000 mg min/cu m was established.[26] Estimates of 44,000 and 35,000 mg min/cu m for DM disseminated from the M6A1 and No. 113 grenades, respectively, were approved at the same time.

B. Estimated ICt50 for Man.

TM 3-215[14] gives the ICt50 as 22 mg min/cu m for a 1-min exposure and 8 mg min/cu m for a 60-min exposure.

From data on the human exposures reported by Gongwer and coworkers,[10] the ICt50 was estimated to be 100 to 350 mg min/cu m for exposure periods of 0.5 to 2 min.

Table XX. Summary of Varying LCt50's for DM Inhalation Toxicity

Species	Pure DM			M6A1 grenade	No. 113 grenade
	1918 - 1964	1965	1918 - 1965	1965	1965
	mg min/cu m				
Monkey	11,756 (6,686 - 19,023) 3.0	17,837 (15,351 - 20,725) 12.5	13,866 (10,984 - 17,235) 4.0	19,569 (14,193 - 26,980) 3.5	22,814 (16,297 - 31,936) 5.2
Dog	17,809 (13,700 - 23,732) 3.4	7,888 (5,951 - 10,457) 5.6	13,945 (10,857 - 18,249) 2.7	28,193 (22,673 - 35,212) 7.2	28,428 (21,633 - 37,376) 1.7
Swine		56,364 (16,709 - 190,140) 2.4	56,364 (16,709 - 190,140) 2.4	36,911 (12,202 - 111,530) 2.1	35,888 (28,854 - 44,637) 9.9
Goat		12,135 (8,051 - 18,292) 4.4	12,135 (8,051 - 18,292) 1.3	8,076 (945 - 69,016) 1.7	11,723 (5,335 - 25,763) 2.2
Rabbit		2,903 (No limits) 1.9	2,903 (No limits) 1.9	41,159 (7,645 - 221,577) 1.9	46,959 (39,615 - 55,665) 5.2
Rat	14,045 (8,473 - 36,383) 0.7	19,234 (17,924 - 20,646) 12.0	12,710 (9,636 - 17,871) 1.0	66,856 (64,033 - 69,804) 3.8	48,217 (42,489 - 54,718) 3.8
Guinea pig	9,906 (6,420 - 20,093) 0.9	4,623 (3,391 - 6,303) 2.2	6,599 (5,087 - 8,909) 1.3	12,591 (12,155 - 13,042) 3.3	29,888 (26,615 - 33,562) 4.6
Mouse	46,245 (16,617 - 3,801,791) 0.6		46,245 (16,617 - 3,801,791) 0.6		
All rodents	16,179 (10,996 - 26,929) 0.7	10,951 (8,397 - 14,282) 1.8	11,769 (9,451 - 15,233) 1.0	83,380 (6,125 - 431,143) 1.0	37,980 (34,593 - 41,699) 3.3
Nonrodents	15,351 (12,307 - 19,401) 3.0	10,233 (5,996 - 17,465) 1.4	13,280 (10,800 - 16,030) 2.0	24,462 (24,277 - 24,648) 2.0	30,063 (25,846 - 34,995) 3.0
All species	15,052 (11,041 - 22,941) 0.7	12,306 (10,283 - 14,726) 2.0	11,309 (9,546 - 13,600) 1.0	43,898 (24,549 - 78,178) 1.0	34,683 (30,245 - 39,773) 3.0
No. of animals	868	407	1,275	473	656

Note: Slope value is given in each instance under the LCt50 value.

A letter from COL J. Batte to CG, USAMUCOM[25] endorsed the CRDL estimate of 150 mg min/cu m for inhaled DM.

C. Estimated ICt50 for Systemic Effects.

None of the available data are adequate to establish an ICt50 for systemic effects (nausea, vomiting, etc).

D. Safety Factors for Inhaled DM in Man.

Based on the relationship between the estimated LCt50's for DM dispersed by various methods and the ICt50 of 22 to 150 mg min/cu m, the safety factors for inhaled DM in man are shown in table XXI.

Table XXI. Safety Factors for Inhaled DM in Man

System	LCt50	ICt50	Safety factors (LCt50/ICt50)
	mg min/cu m	mg min/cu m	
Pure DM (Based on all experiments performed 1918 - 1965)	11,000	22 - 150	500 or 73
M6A1 grenade (1965)	44,000	22 - 150	2,000 or 293
No. 113 grenade (1965)	35,000	22 - 150	1,590 or 234

V. SUMMARY.

A. Incapacitating Effects of DM in Man.

The onset of signs caused by DM may be almost immediate or delayed for several minutes. The initial effects are irritation, a burning sensation and pain in the eyes, nose, throat, and respiratory tract, uncontrollable cough, violent and persistent sneezing, lacrimation, and copious flow of saliva. The conjunctiva, nose, and pharyngeal wall become congested. The signs of irritation subside after 20 to 30 min. Headache, depression, perspiration, chills, nausea, abdominal cramps, vomiting, and diarrhea may appear in about 30 min after exposure and persist for several hours.

A dose-effect graph for intolerable concentrations of DM was developed by Lawson and Temple in 1922.[6] It included concentrations of 22.3, 0.7, 0.2, and 0.14 mg/cu m for exposure periods of 1, 5, 15, and 60 min, respectively. In this test, an alcoholic solution of DM was dropped into a heated tube, and the cloud produced was conveyed into a mixing chamber by a stream of nitrogen. The men breathed the cloud through a 1919-type mask connected to the chamber by a three-way valve. The concentrations of DM were estimated nominally. Subjects were told to keep the mask on until there was a feeling of distress, but because of the nature of the gas, they were not expected to fight it to the limit of their endurance.

Results of field tests during the early 1920's[6] indicated that some subjects tolerated DM at Ct's of 83 to 155 mg min/cu m. Although the quantitative aspects of these field exposures are somewhat doubtful, there is a discrepancy between the intolerable doses repeated by Lawson and Temple[6] and those measured in the field.

Other human exposures at CRDL in 1958 indicated that men could tolerate concentrations of 22 to 92 mg min/cu m for 1 min or more. The higher value resulted when the subjects were told to resist the agent.

B. Systemic Effects.

An important consideration concerning DM is its persistent incapacitating effects, including malaise, depression, nausea, and vomiting. However, the dose required to produce these effects and the frequency of occurrence of these signs are a matter of question. In the studies conducted in 1922,[6] nausea occurred in 3 of 21 men at concentrations of 2 mg/cu m after they had previously been exposed to a concentration of 4 mg/cu m for periods of 45 sec to 12-1/2 min (Ct's of 3 to 50 mg min/cu m).

Lawson and Temple[6] indicated a low frequency of systemic effects in their studies. "Delayed effects were infrequent, an occasional dull headache persisting for several hours, and in one case, where the concentration was 0.06 mg/liter (60 mg/cu m) a man was incapacitated for work for 2 days with stomach trouble, dull headache, and general depression." A few other cases were found where stomach trouble occurred. In the writer's opinion, this was caused by gas due to individual susceptibility.

In the human studies conducted in 1958, systemic effects were seen infrequently. Nausea was experienced by two men exposed to Ct's of

18 and 22 mg min/cu m. An additional 18 men exposed to Ct's ranging from 22 to 144 mg min/cu m and 5 men exposed to Ct's of less than 22 mg min/cu m had no systemic effects. These data are not adequate to establish an ICt50 for systemic effects.

C. Lethality of DM in Man.

One death has been attributed to inhalation of DM. This followed the operation of a DM generator in a barrack exposing 22 sleeping men. The estimated concentration was 1,130 to 2,260 mg/cu m. The exposure period was 5 or 30 min, according to different reports. The Ct's would be 5,650 to 11,300 mg min/cu m for the 5-min exposure and 33,900 to 67,800 mg min/cu m for the 30-min exposure.

Post-mortem examination of the victim revealed emphysema of the subcutaneous tissues of the neck, mediastium, pleura, and pericardium. Emphysematous bullae were scattered over the lungs. The lungs were springy and crepitant. Bluish patches appearing to be bronchopneumonia were noted. No consolidation, edema, or casts in the bronchi were noted when the lung was cut.

Histological study showed edema and congestion of the epiglottis, superficial ulceration and acute diffuse inflammation of the trachea and bronchi, false membrane formation in the trachea and bronchi, lung congestion, edema, hemorrhage, and bronchopneumonia.

The cause of death following inhalation of DM by man can be attributed to damage to the lungs and respiratory system.

D. Toxicity Studies of DM in Animals.

One of the striking features of DM inhalation toxicity studies is the variation in results of different experiments. The British Red Book[12] declined to quote toxicity values for this compound in animals because of the inconsistencies in results. Possibly, the methods of dispersion of the aerosols and the methods of measuring airborne concentrations contributed to the variabilities.

The data used in this report to determine the toxicity of DM when aerosolized by various methods include dispersions of molten DM to dogs (1918)[4]; dry dust dispersions to mice, rats, and guinea pigs (1957); acetone

dispersions to mice, rats, guinea pigs, dogs, and monkeys (1963 - 1964); and acetone and munition, M6A1 and No. 113 grenades, dispersions to rats, guinea pigs, rabbits, dogs, monkeys, swine, and goats. The LCt50 values for each experiment and combinations of the LCt50 values for laboratory dispersion methods and munition disseminations are shown in tables X through XV.

E. Toxicological Signs in Animals.

The signs were similar for all types of dispersions and were as follows for animals receiving lethal and sublethal inhalation dosages.

1. Mice, Rats, and Guinea Pigs.

Immediately upon exposure, the animals were hyperactive. Within a few minutes, lacrimation and salivation were observed. After 5 to 15 min, the excitement was generally supplemented by lethargy and labored breathing. The latter signs often persisted for 1 or 2 hr after exposure. The other signs usually subsided within 5 to 10 min after the animals were removed from the contaminated atmosphere.

2. Dogs.

Immediately upon exposure, the dogs became extremely restless. Jumping and barking were noted. Salivation, retching, and vomiting occurred. The animals became ataxic, and some were unable to maintain a standing posture. Upon removal from the chamber, they were hypoactive and pawed their faces. Gagging and vomiting occurred periodically for 24 hr. They consumed little food or water and, for about 7 days, they appeared emaciated. After 7 days, the animals resumed normal eating and drinking and improved in appearance. Most deaths occurred in the first week after exposure.

3. Monkeys.

During exposure, salivation, vomiting, rhinorrhea, ataxia, and difficulty of breathing were noted. Upon removal from the chamber, the animals exhibited wheezing, ptosis, and lethargy. Coughing and vomiting persisted for about 24 to 48 hr. After 24 to 48 hr, open lesions were noted on the face and around the eyes, possibly due to pawing by the animal. Prior to death, the monkeys lay face down, and their breathing was depressed.

4. Goats.

Signs that occurred during exposure were hyperactivity, shaking of the head, rearing, licking, chewing, frothing at the mouth, ataxia,

convulsions, bloating, and death. During the week following exposures, the animals were hypoactive, knelt on their forelegs, gagged, and vomited. The goats seemed weak; they collapsed and convulsed prior to death. All goats were bloated upon death.

5. Swine.

The signs noted during exposure were salivation, frothing at the mouth, ataxia, and irregular breathing. During the first 14 days after exposure, the pigs had breathing difficulty, lost weight, appeared emaciated, and some died.

F. Toxic Doses for DM.

The combined data for pure DM (dry dust, molten agent, and solvent dispersion) in 1,273 animals (mice, rats, guinea pigs, dogs, monkeys, swine, and goats) exposed from 1918 to 1965 yield an LCt50 of 11,309 mg min/cu m.

The combined LCt50 for seven species exposed to DM in acetone dispersed as a spray during studies performed in 1965 was 12,306 mg min/cu m.

Combined LCt50's for 473 animals (rats, guinea pigs, rabbits, dogs, monkeys, swine, and goats) exposed to DM disseminated from the M6A1 grenade and for 656 animals (same species) exposed to DM disseminated from the No. 113 grenade were 43,808 and 34,683 mg min/cu m, respectively.

G. Repeated Exposures to DM.

Monkeys, dogs, and guinea pigs were exposed to DM aerosols (No. 113 grenade) on 10 consecutive days. The daily doses were approximately at the LCt5 level. A similar group of animals was exposed to approximately the LCt20 to 25 level on each of 10 days. In both cases, the accumulated doses would be expected to kill all animals if the total dose were given in a single exposure.

The lower dose level killed five out of eight monkeys. This is more than would be expected from any one of the exposures alone, but less than would be expected from the total accumulated dose. The deaths among the

dogs and guinea pigs at the low dose level were less than would have been expected from any of the single exposures and far less than would be expected from the accumulated dose.

The deaths in monkeys and guinea pigs at the higher dosage level are slightly greater than what would have been expected for the greatest single dose. The deaths in dogs were less than what would have been expected of the greatest single dose. There was little indication of cumulative toxicity due to the repeated exposures.

H. Local Application of DM to Rabbit Eyes and Skin.

A suspension of DM in corn oil was administered intraocularly to groups of six rabbits each at doses of 0.1, 0.2, 0.5, 1.0, and 5.0 mg per eye. All animals were observed for 14 days after dosing. A dose of 0.1 mg produced no noticeable signs; 0.2 mg produced a transitory conjunctivitis; 0.5 mg caused a transitory conjunctivitis and blepharitis; 1.0 and 5.0 mg produced corneal opacity, which persisted during the 14-day period.

Suspensions of DM in corn oil were placed upon the clipped skin of rabbits. Doses of 1, 10, 50, 75, and 100 mg per animal were administered to groups of six rabbits each. Doses of 10 mg and above produced necrosis.

I. Pathological Findings Following Inhalation of DM in Animals.

Pathological findings in animals that died following inhalation of DM include the following: (1) Dogs — hyperemia of the larynx and trachea, edema and congestion of the lung, and bronchopneumonia; (2) rats and mice — atelectasis, emphysema, reticular cell proliferation, respiratory epithelial proliferation, and interstitial leucocytic infiltration of the bile duct; (3) monkeys — pneumonitis, ulcerative bronchiolitis and tracheitis, and edema and congestion of the lungs; and (4) guinea pigs — bronchitis and tracheitis.

The primary cause of death in animals was lung damage.

J. LCt50 Doses of DM for Man.

An estimate for the toxicity of inhaled DM in man was established at CRDL in 1959. This estimate used toxicity data on mice and guinea pigs reported in Tech Memo 24-18[10] and data on dogs reported in EACD 145.[9]

All of the toxicity data were combined to yield a composite lethality dose-response graph for mammals including man. The LCt50 for a single exposure was 14,000 mg min/cu m.

More recent studies have greatly increased the number of animals and species. The combined LCt50's for pure DM (dispersed as molten agent, dry dust, or from solvent) in mice, rats, guinea pigs, dogs, monkeys, swine, and goats was 11,000 mg min/cu m. In these experiments 1,270 animals were exposed.

The combined LCt50's for DM dispersed from the M6A1 grenade and the No. 113 grenade in mammals are 44,000 and 35,000 mg min/cu m, respectively. Until 1965 no DM munition had been studied for inhalation toxicity. The toxicities are similar for the two munitions, and both produce aerosols that appear less toxic than those produced from pure DM.

K. Safety Factors for Inhaled DM.

On the basis of data presented in this report, the best safety factors that apply to DM dispersed by various methods are given in table XXI.

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APPENDIXES

APPENDIX A

METHODOLOGY

The following section describes the experimental methods used by three laboratories whose animal inhalation toxicity studies with DM are reported.

Laboratory No. 1 - War Department, Chemical Warfare Service, Research Division, American University Experimental Station, Washington, D. C.; investigators, C. A. Ransom and F. B. Bogart. The following is quoted verbatim from their report.[1]

> Dispersion - The substance was aerated in a flask which was heated to 210°C in paraffin bath. The method was controlled by chemical analysis.
>
> Observations - The animals were observed for signs during and after exposure. Times of death was noted. Pathological examination was performed on dogs which died and also on animals which were sacrificed at 9, 12, 14 or 15 days after exposure.

Laboratory No. 2 - Hazleton Laboratories, Falls Church, Virginia; investigators, J. Mennear, H. Jennings, D. McCarthy, H. Bolden, J. Ott, B. Smith, and P. Warman. The following is quoted verbatim from Hazleton Laboratories Contract Report DA18-108-AMC-78(A).[2]

> Particle size analysis was performed according to the method of May[3] using the Casella Cascade Impactor for collection of particles. The impactor was connected to the sampling tube in the chamber and the particles were optically sized by the comparison method. This method involved the use of the Porton graticule, a modification of the Fairs[4] graticule. A minimum of 400 particles were evaluated under a microscope fitted with a 10X ocular and having a magnification of 44X.

Laboratory No. 3 - Aerosol Branch, Toxicology Division, Directorate of Medical Research, Edgewood Arsenal, Maryland; investigators, E. J. Owens, C. L. Punte, J. T. Weimer, T. A. Ballard, J. T. Hiddemen,

W. E. Hickman, R. L. Farrand, T. L. Hess, G. F. Egan, C. F. Hoffman, W. U. Thomas, S. G. Ryan, S. F. Sell, J. S. Olson, R. P. Merkey, J. Burns, and W. M. Lawson; 1957 - 1965.

1. Materials.

a. Dry Dust or Acetone Dispersions.

All experiments conducted between 1957 and 1965 with DM disseminated by laboratory methods, either at Edgewood Arsenal or at Hazleton Laboratories and whether the agent was aerosolized as a dry dust or from solvent sprays, were performed with a multiton quantity of DM manufactured in 1943. All DM samples used from this lot for the 1965 acetone-spray experiments were analyzed by ultraviolet (UV) spectrophotometry and found to be 95% pure.

b. M6A1 Thermal Grenades.

The M6A1 munitions used in the experiments performed in 1965 were loaded at Pine Bluff Arsenal in July 1964 and designated as Lot No. 1021-51-1001. The grenades were a pilot production lot containing only DM and pyrotechnic fuel. The chemical contents of each canister weighed 130 gm. and consisted of:

43% DM (95% purity)
19% sugar
32% potassium chlorate
5% magnesium oxide
1% magnesium carbonate

The DM used in these grenades was from the multiton 1943 production lot.

c. No. 113 Federal Spedeheat Grenades. The sickening gas (DM) grenades, manufactured by Federal Laboratories, Saltsburg, Pennsylvania, were loaded during June and July 1965. According to verbal information furnished by representatives of the manufacturer, each grenade contained 92.7 gm of DM. The exact formulation of the grenades would not be divulged by the manufacturer. No information could be obtained concerning the purity of the DM used by the Federal Laboratories.

In July 1964, the Amcel Propulsion Co. recrystallized a portion of the 1943 production lot. The purity of this material, determined by UV spectrophotometry, was 97%. The material was used at Edgewood and at Pine Bluff Arsenals in the manufacture of pilot production lots of M6A1 grenades.

In 1965, the Amcel Propulsion Co. synthesized a 100-lb lot of DM. The purity of this sample was greater than 97%. This material was also used at Edgewood Arsenal to fill a small lot of M6A1 grenades.

At the present, neither of these Amcel samples has been tested for toxicity.

2. Animals Used From 1957 to 1965.

a. Mice, Rats, and Guinea Pigs.

All mice, rats, and guinea pigs used by the Aerosol Branch during this period were raised in the Edgewood Arsenal Animal Colony and were from the same genetic strains.

b. Rabbits.

Rabbits were purchased from animal dealers and quarantined in the animal facilities for 3 wk prior to their use.

c. Monkeys.

Upon arrival at the animal facilities, all monkeys were placed in individual cages, tested for tuberculosis, and examined for worms and fecal parasites. All monkeys were quarantined for a minimum of 6 wk prior to experimental use. Since early 1965, dealers furnishing monkeys to Edgewood Arsenal have had to guarantee that the animals had been housed in the US for at least 1 mo for observation purposes.

d. Dogs (Mongrels).

All dogs were supplied by animal dealers. Upon arrival, they were weighed; examined for distemper, hepatitis, and leptospirosis; immunized against rabies; dipped for external parasities; checked for worms and fecal parasities; and quarantined for a minimum of 3 wk prior to experimental use.

e. Swine and Goats.

The swine used in all experiments were Doroc-Jersey pigs, weighing approximately 30 to 40 lb; they were supplied by various dealers.

The goats were of a nonspecific dairy strain, weighing approximately 30 to 40 lb; they also were supplied by various dealers.

3. Aerosol Exposure Techniques From 1957 to 1965.

From 1957 to 1964, the exposures were conducted in chambers of various sizes and shapes as follows:

20-ℓ Bell jar

100-ℓ plastic rectangular chamber

1,000-ℓ cube chamber

250- and 1,000-ℓ hexagonal chamber (containing radial perforated tubes and plates at the top and bottom to distribute the air evenly and to prevent channeling of aerosol or vapor).

These chambers are shown schematically in figure A-1.

The chamber used for the DM acetone spray in 1965 is a 20,000-ℓ cylinder with concave top and bottom. The height of the side walls is 8 ft, and the height from the centers of the concave top and bottom is 11 ft. The diameter of the cylinder is 10 ft. This chamber contains radial perforated distribution tubes and plates at the top and bottom to lead the aerosol into and out of the chamber. The tubes aid in evenly distributing the aerosol cloud.

All exposures in these chambers were conducted with dynamic clouds. The details of chamber calibration and operation are described by Silver,[5] Vocci and coworkers,[6] Punte and coworkers,[7] and Weimer and coworkers.[8]

Exposures of animals in the dynamic chambers were as follows. The bell jar was used to expose up to four rats or four guinea pigs per test and was operated at flow rates of less than 20 ℓ/min. In the 100-ℓ rectangular chamber, six rats, six guinea pigs, or 20 mice or less were exposed

at one time. The flow rate was approximately 50 ℓ/min. The 1,000-ℓ cube was used to expose up to four dogs per test and was operated at flow rates of 500 to 1,000 ℓ/min. The 250-ℓ hexagonal chamber was used to expose up to 20 rats or guinea pigs per test. The flow rate in this chamber was from 125 to 250 ℓ/min. The 1,000-ℓ hexagonal chamber was used to expose up to six dogs or monkeys. The flow rates in this chamber were 500 to 1,000 ℓ/min. In the 20,000-ℓ cylinder, groups of 20 rats, 20 guinea pigs, six dogs, and six monkeys were exposed at the same time. Groups of six pigs and six goats were exposed together. The flow rate in this chamber was about 10,000 ℓ/min.

Acetone solutions containing up to 10% DM were forced by compressed air through various types of atomizers into a constant-flow airstream that flowed into and through the exposure chamber. In some of these exposures, the airborne concentration of DM was kept relatively constant, and the exposure times were varied to produce different Ct levels. In some exposures, both concentrations and exposure times were varied to produce different Ct levels. In other exposures, both concentrations and exposure times were varied.

The cloud was sampled for chemical analysis periodically during the exposure period.

In the munition experiments conducted in 1965, a 20,000-ℓ cylindrical chamber, measuring 13.5 ft in height and 8 ft in diameter, was used. The cloud was mixed by turbulence and diffusion and was maintained statically during the animal exposure. Groups of 20 rats, 20 guinea pigs, six dogs, six rabbits, and six monkeys were exposed simultaneously. Groups of six pigs and six goats were exposed together. Samples of the cloud were taken and chemically analyzed continuously during short exposures of several minutes or periodically during the longer exposures. After a given time, the chamber was cleared within 2 min by evacuating the cloud into the dynamic aerosol wind tunnel.

In all of the 1965 studies (and in many of the earlier studies), there were Ct levels that produced no deaths, lesions, or biochemical changes. These may be considered as controls on the procedures involved. The same procedures involving other compounds of negligible toxicity give strong indication that the exposure procedures have no effect on animals.

4. Determination of Particle Size.

Determinations of the aerosol particle sizes produced by 10% acetone sprays, M6A1 grenades, and the No. 113 grenades were made at various times during animal exposures to the three systems. Samples for these determinations were taken with a modified Rochester cascade impactor. The mass median diameter (MMD) was derived by the use of stage calibrations based on the density of each compound. The results of these determinations are shown in table A-I.

5. The Chemistry and Bioassessment of DM. *

Prior to the advent of infrared (IR) spectrophotometry, there were no analytical procedures that were specific for the DM molecule. In the period before 1957, toxicological experiments did not present information on the methods used in analyzing the cloud for DM. From 1957 until the present time, UV spectrometry had been used most frequently to determine the purity of DM samples and to determine the concentration of DM in airborne dispersions. An IR spectrophotometer became available in the Aerosol Branch in early 1965. This technique has been used to check the UV method.

The IR studies were conducted after it was found that the pure DM and the DM that was carbonate cleaned (oxidized) appeared to have different UV spectra. The solvent used for these studies was carbon disulfide, and in every series, the spectra were those of CS_2 versus CS_2. The other spectra (B, C, and D in figures A-2 to A-7) were those of sample weights that would be equal to 1, 2, and 3 mg/ml of pure DM. The purity of each sample was determined by UV analysis.

Figure A-2 consists of spectra for bicarbonate-neutralized DM, where A = CS_2 versus CS_2, B = 1 mg/ml in CS_2 versus CS_2, C = 2 mg/ml in CS_2 versus CS_2; figure A-3 consists of spectra for pure DM, where A = CS_2 versus CS_2, B = 1 mg/ml in CS_2 versus CS_2, C = 2 mg/ml in CS_2 versus CS_2, and D = 3 mg/ml in CS_2 versus CS_2.

These spectra show the difference in the IR analysis of the two compounds that gives like curves when analyzed by the UV method. The neutralization and oxidation of the DM molecule show a great difference

* Vocci, F. J., and Feinsilver, L. Toxicology Division, Aerosol Branch and Basic Toxicology Branch.

Table A-I. Particle-Size Determinations of DM Acetone Spray, M6A1 and No. 113 Grenade Dispersions, and a Statistical Analysis of These Data

Dispersion system	No. of tests	Stage calibrations		Mass distribution	Percent of mass	Cumulative percent of mass	Statistical analysis				
		No.	Particle size				P	ED(P)	Lower limit	Upper limit	SE of slope
			μ								
DM spray (10% in acetone)	5	I	10.9	17	2.6	100.0	1	0.221	0.210	0.233	0.23
		II	4.6	136	21.1	97.4	16	0.576	0.561	0.591	
		III	2.3	141	21.9	76.3	30	0.809	0.795	0.822	
		IV	1.3	102	15.8	54.4	50	1.186	1.172	1.196	MMD = 1.2μ
		V	0.74	242	37.5	38.6	84	2.442	2.386	2.504	
		VI	0.41	7	1.1	1.1	99	6.492	6.160	6.862	
M6A1	6	I	10.9	8	0.5	100.0	1	0.218	0.206	0.232	0.30
		II	4.6	142	9.4	99.5	16	0.497	0.484	0.511	
		III	2.3	233	15.5	90.1	30	0.669	0.657	0.681	
		IV	1.3	368	24.5	74.6	50	0.936	0.928	0.945	MMD = 0.94μ
		V	0.7	546	36.3	50.1	84	1.797	1.765	1.833	
		VI	0.4	208	13.8	13.8	99	4.477	3.182	4.650	
DM apedeheat No. 113)	3	I	10.9	20	0.1	100.0					
		II	4.6	100	0.8	99.9	No probit possible - monodispersed aerosol system with greater than 95% of the particles <1μ				
		III	2.3	184	1.5	99.1					
		IV	1.3	868	6.8	97.6					
		V	0.7	11,376	90.1	90.7					
		VI	1.41	76	0.6	0.6					

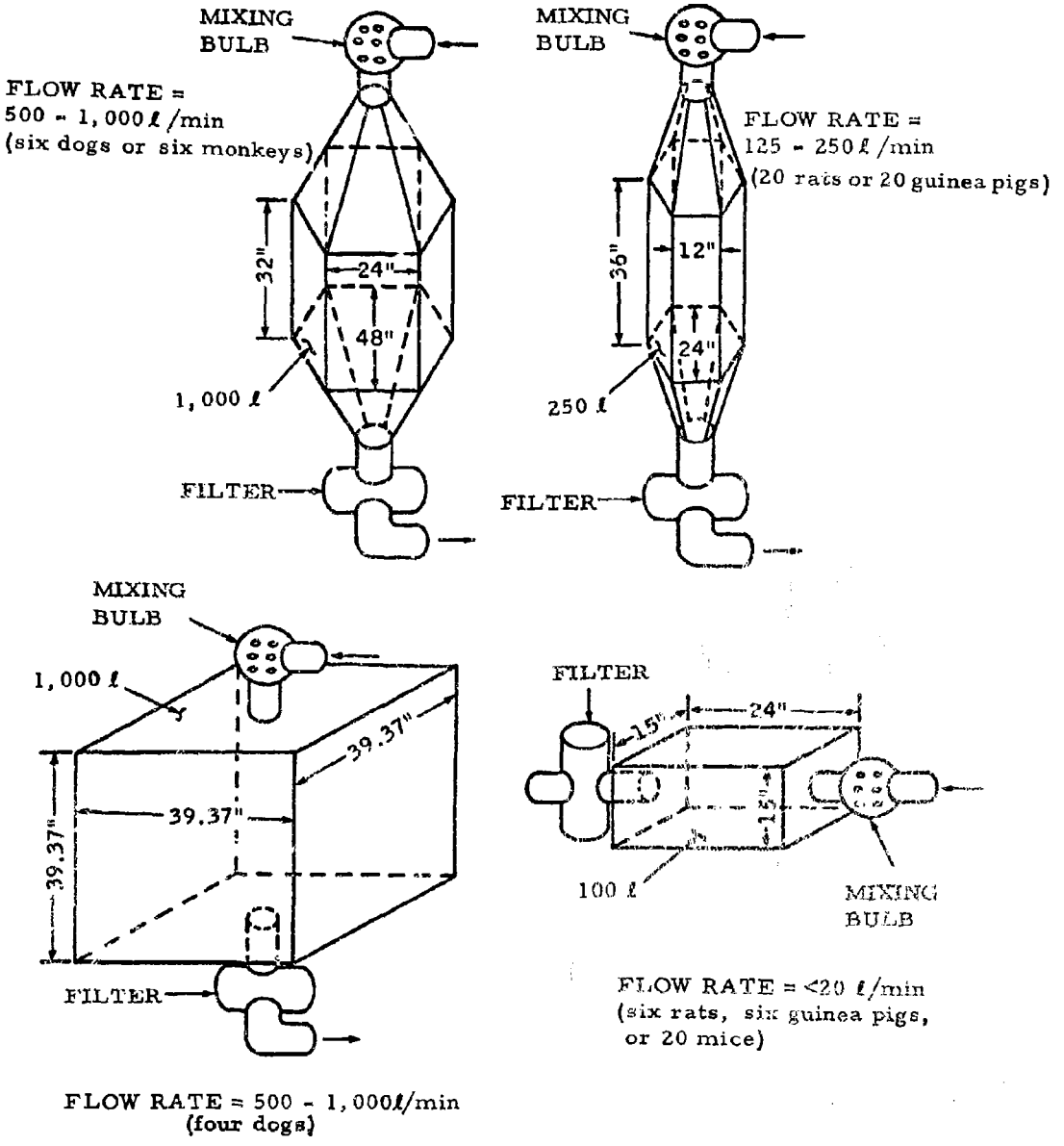


Figure A-1. Schematic Drawings of Aerosol Chambers Used at CRDL for Experiments During 1957 to 1964

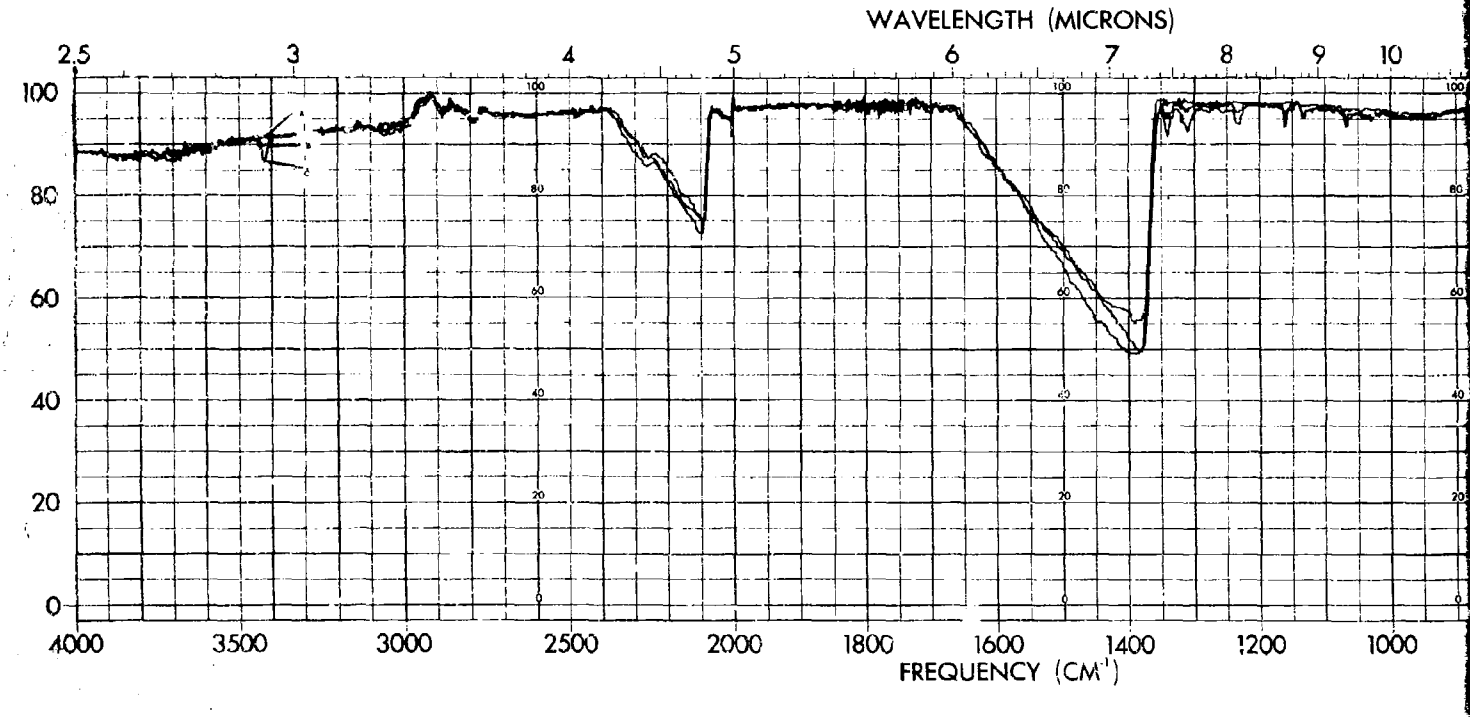
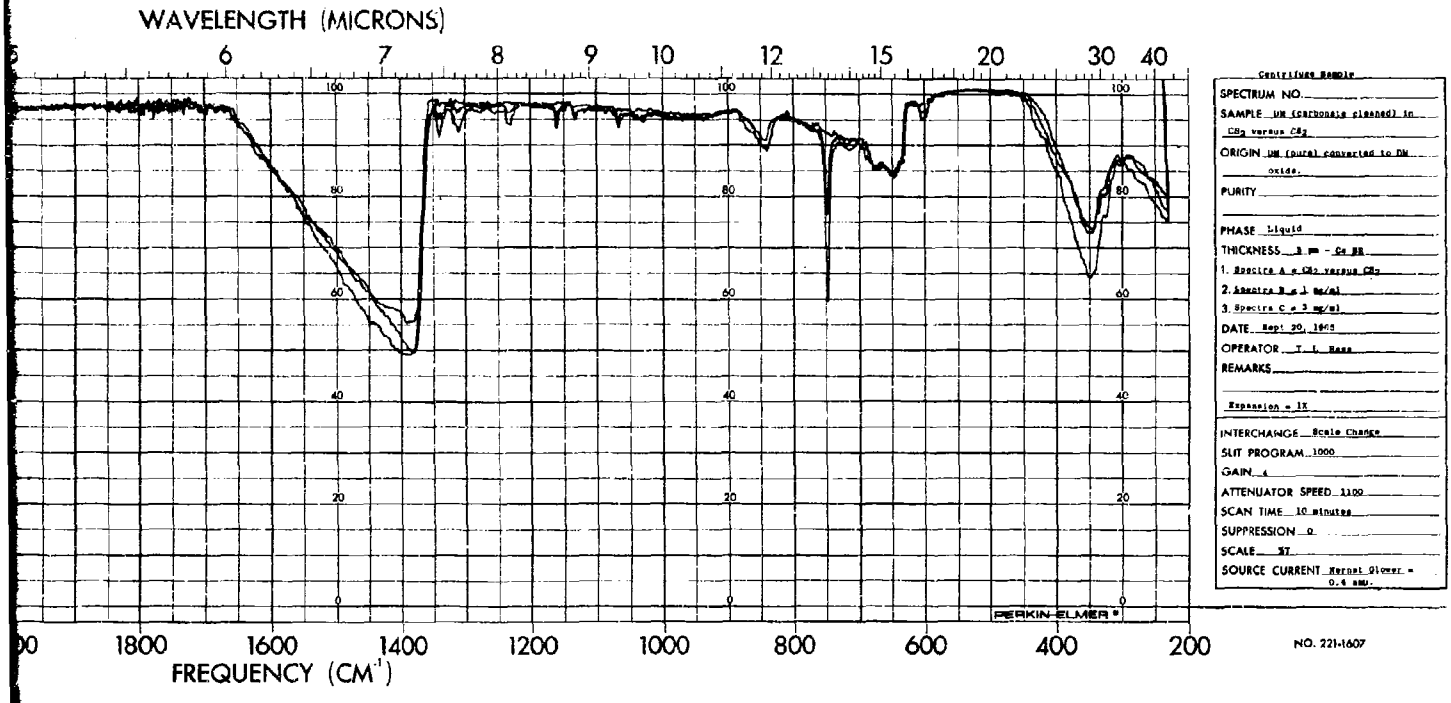


Figure A-2. Spectra for Bicarbonate-Neutralized D



gure A-2. Spectra for Bicarbonate-Neutralized DM

2

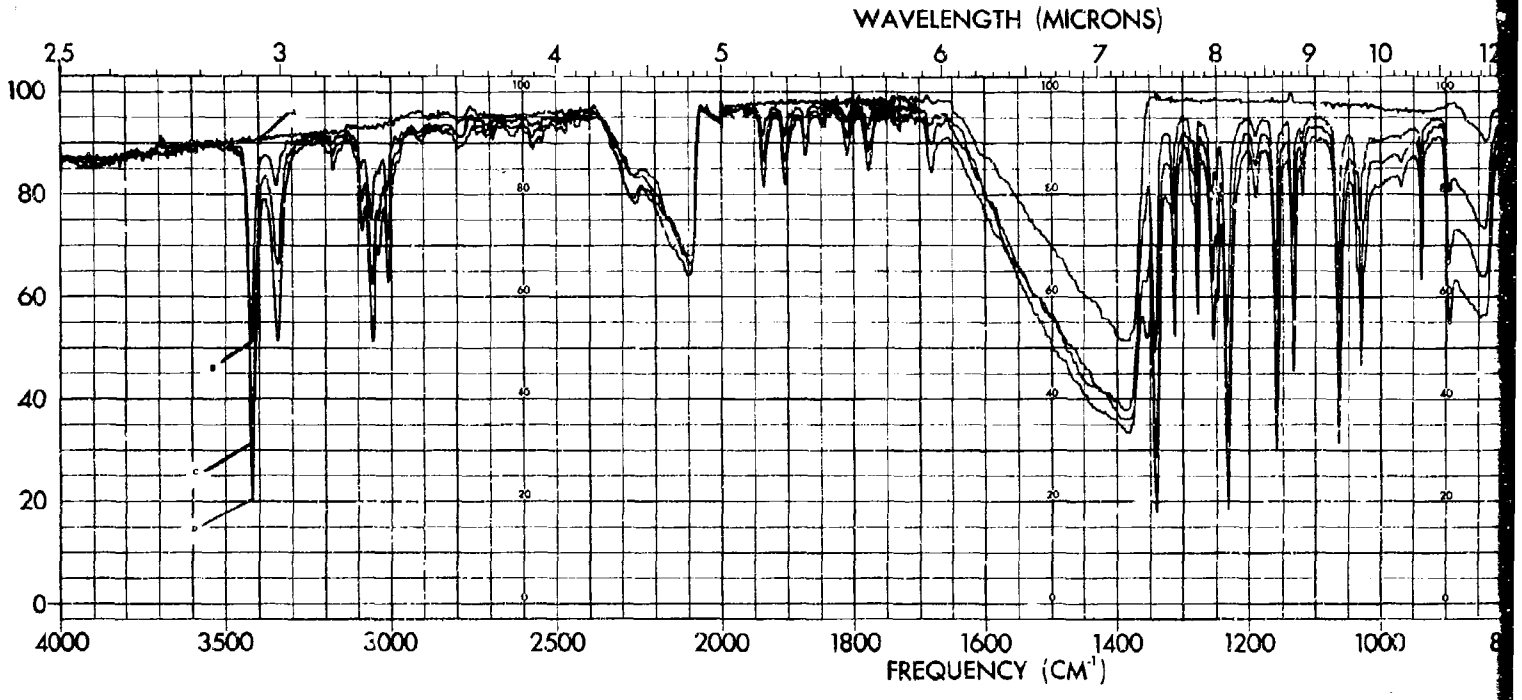


Figure A-3. Spectra for Pure DM

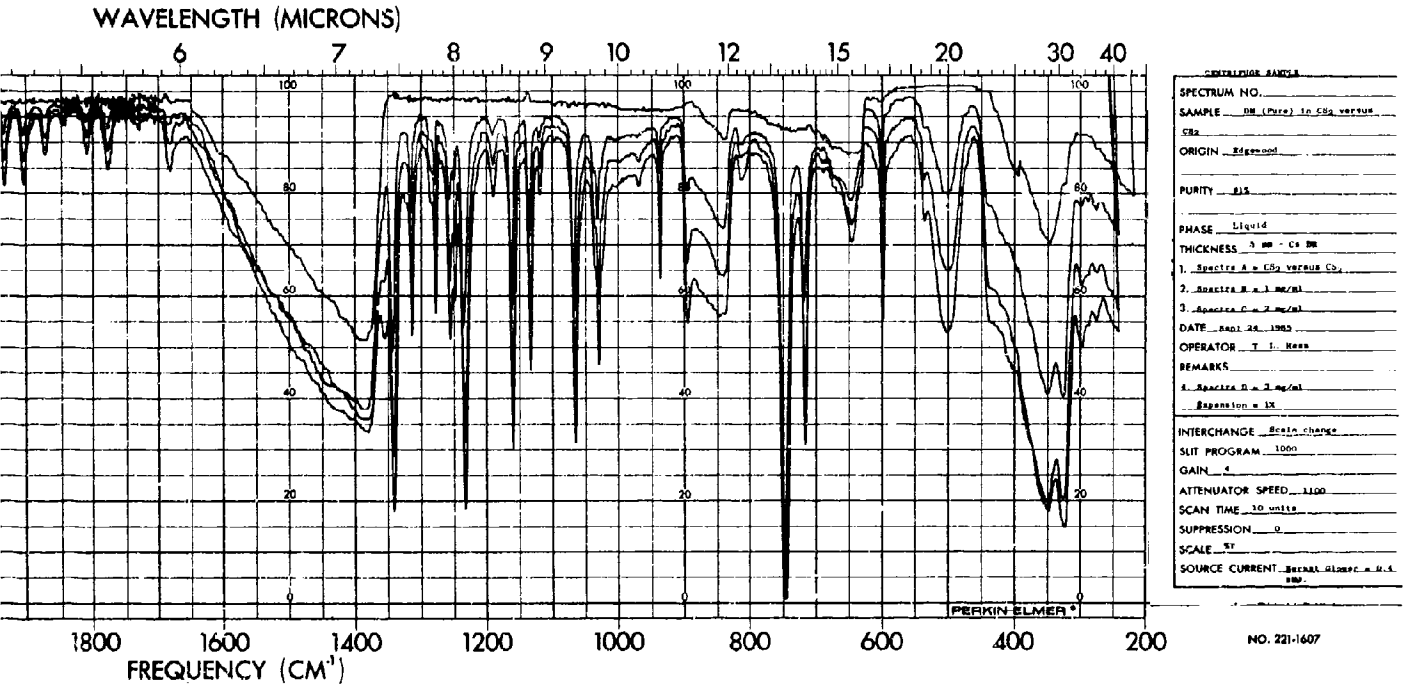


Figure A-3. Spectra for Pure DM

2

at 3,240 cm^{-1} with an almost complete disappearance of this band when compared with a sample of pure DM.

Figure A-4 consists of spectra for DM from a burned M6A1 grenade with weight based on UV analysis of 6.9% DM and corrected to 100%, where A = CS_2 versus CS_2, B = 1 mg/ml in CS_2 versus CS_2, C = 2 mg/ml in CS_2 versus CS_2, and D = 3 mg/ml in CS_2 versus CS_2; and figure A-5 consists of spectra for DM from an opened M6A1 grenade with weight based on 43% DM and corrected to 100%, where A, B, and C are the same as above. These spectra show that a true DM particle is produced and disseminated by the M6A1 grenade.

Figure A-6 consists of spectra for DM from a burned No. 113 grenade with weight based on UV analysis of 25% DM and corrected to 100%, where A = CS_2 versus CS_2, B = 1 mg/ml in CS_2 versus CS_2, C = 2 mg/ml in CS_2 versus CS_2, and D = 3 mg/ml in CS_2 versus CS_2; and figure A-7 consists of spectra for DM from an opened No. 113 grenade with weight based on UV analysis of 25% DM and corrected to 100%, where A, B, C, and D are the same as above.

The main peaks that were compared in the various samples were the peaks at 3,240 cm^{-1} due to N—H bond (unassociated), N—H stretching, and the peaks at 750 cm^{-1} due to the C—H bonding of the orthosubstituted benzene ring.

The preceding analytical studies were conducted with the Perkin-Elmer Model 521 IR spectrophotometer. The cells used were a matched pair of variable cesium bromide cells set at a path length of 5 mm with a spectrophotometer expansion of IX.

When compared with the UV studies, these studies showed that the two methods should be used together when rating the compound.

The reliability of the UV analysis was investigated by studying the following samples of DM: (1) 95% pure DM; (2) 95% pure DM from which the free acid had been removed by washing with a solution of sodium bicarbonate followed by washing with water; (3) 43% DM as contained in the unburned pyrotechnic mixture of M6A1 grenades; (4) DM as contained in the unburned pyrotechnic mixture of the No. 113 grenades; and (5) in combination with the cloud contaminants as disseminated from the No. 113 grenades.

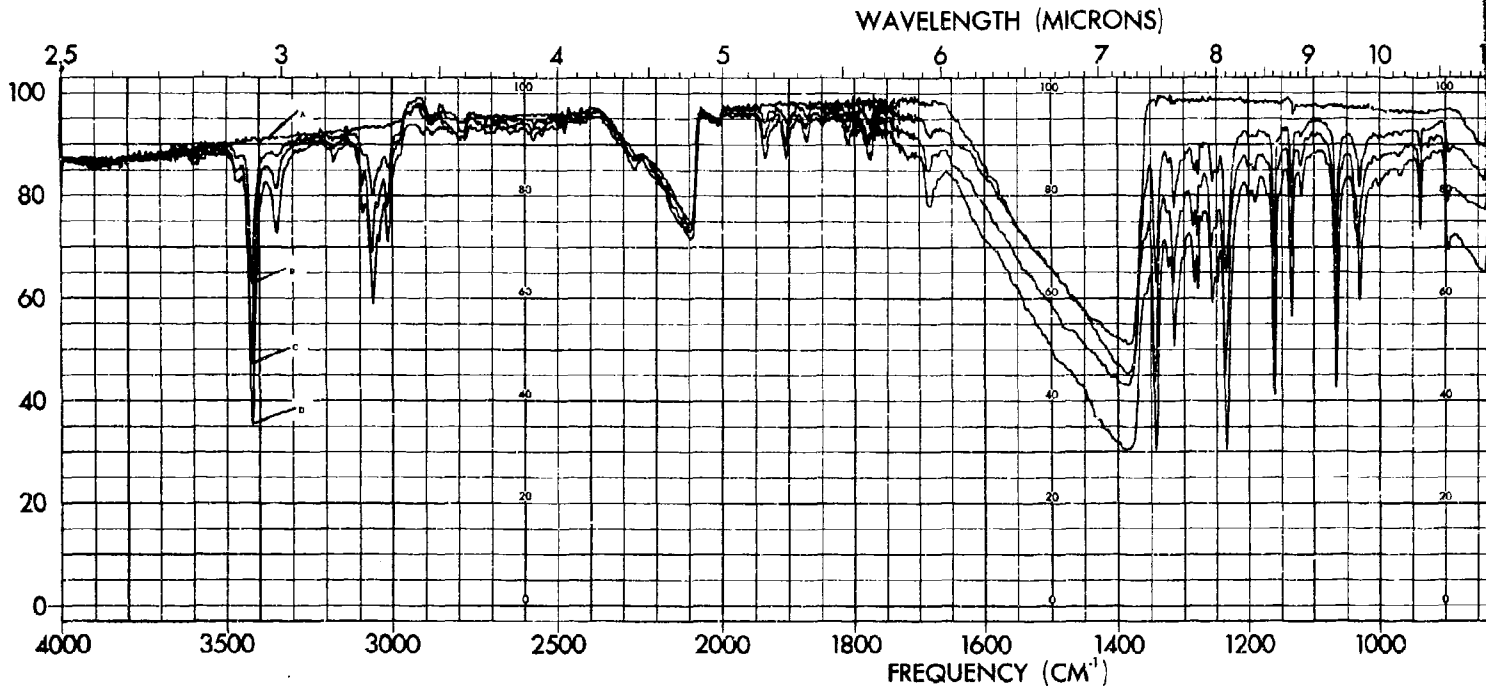
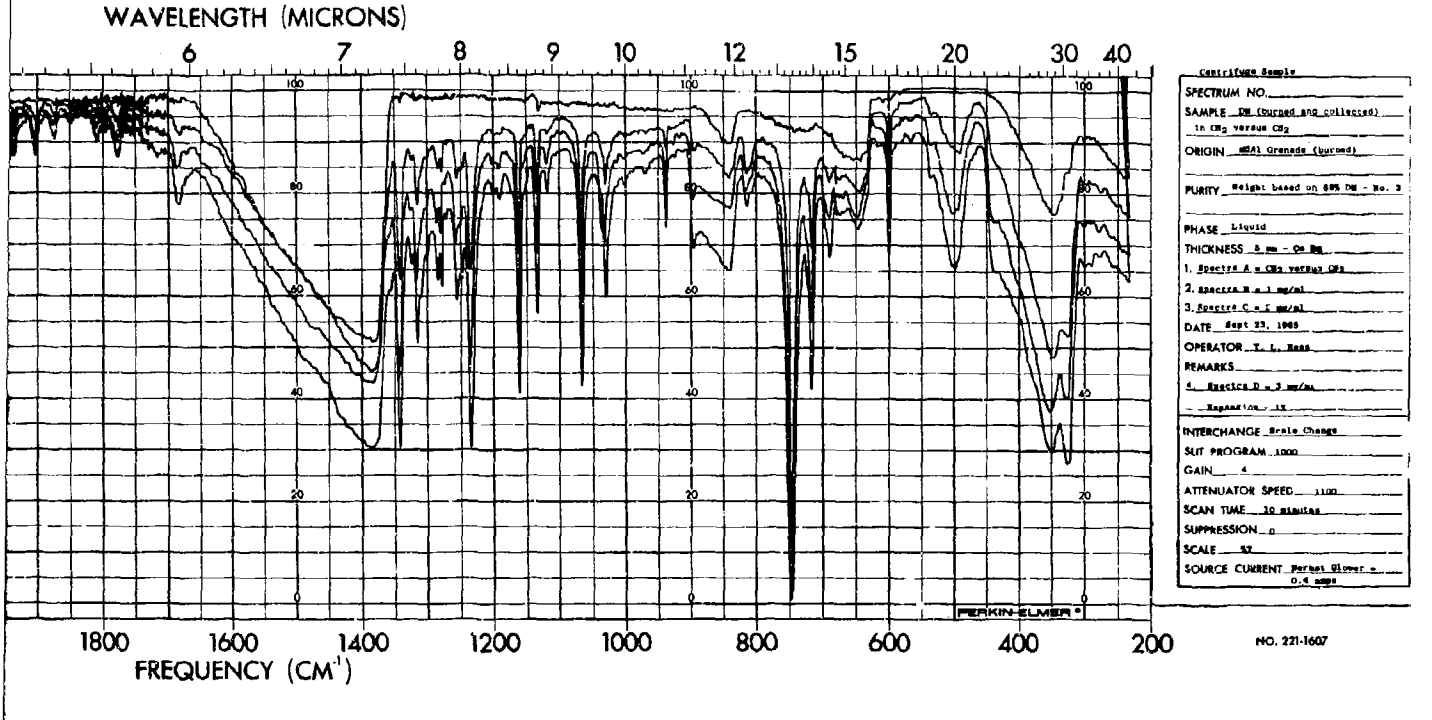


Figure A-4. Spectra for DM From a Burned M6A1 Grena



A-4. Spectra for DM From a Burned M6A1 Grenade

2

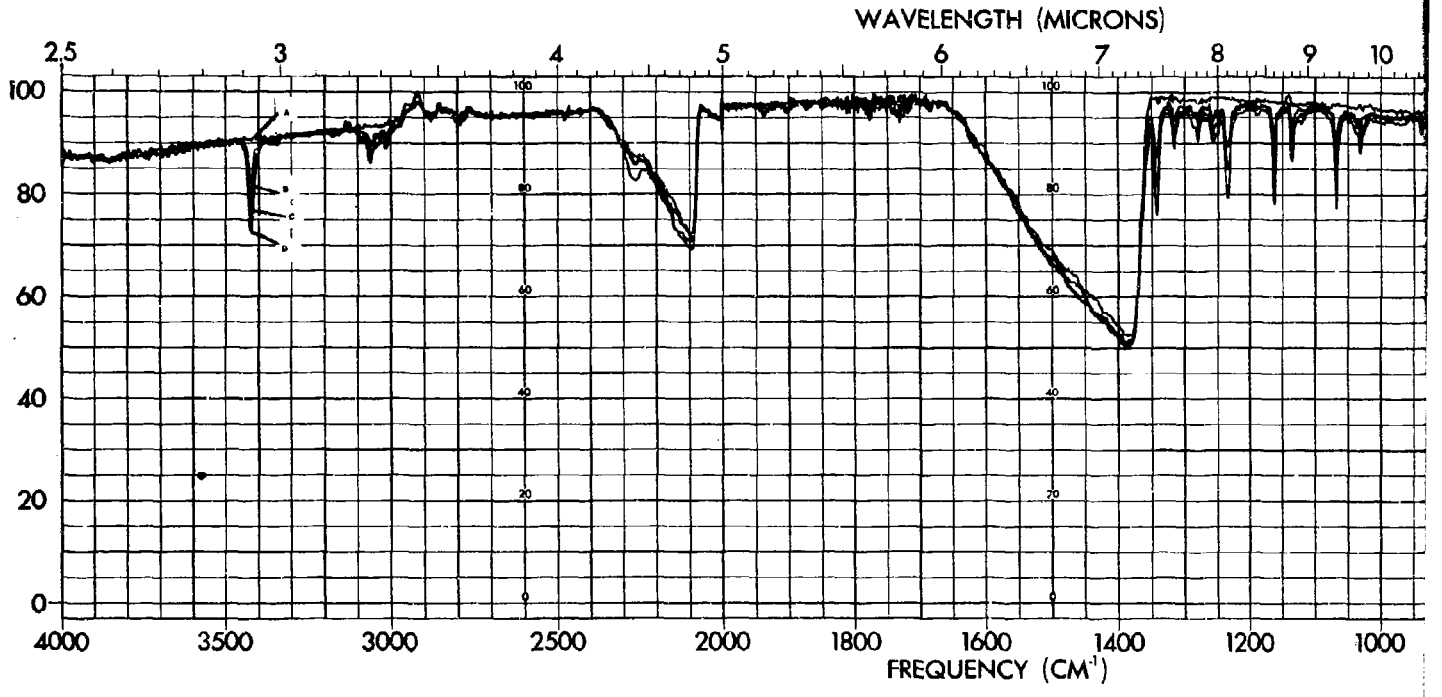
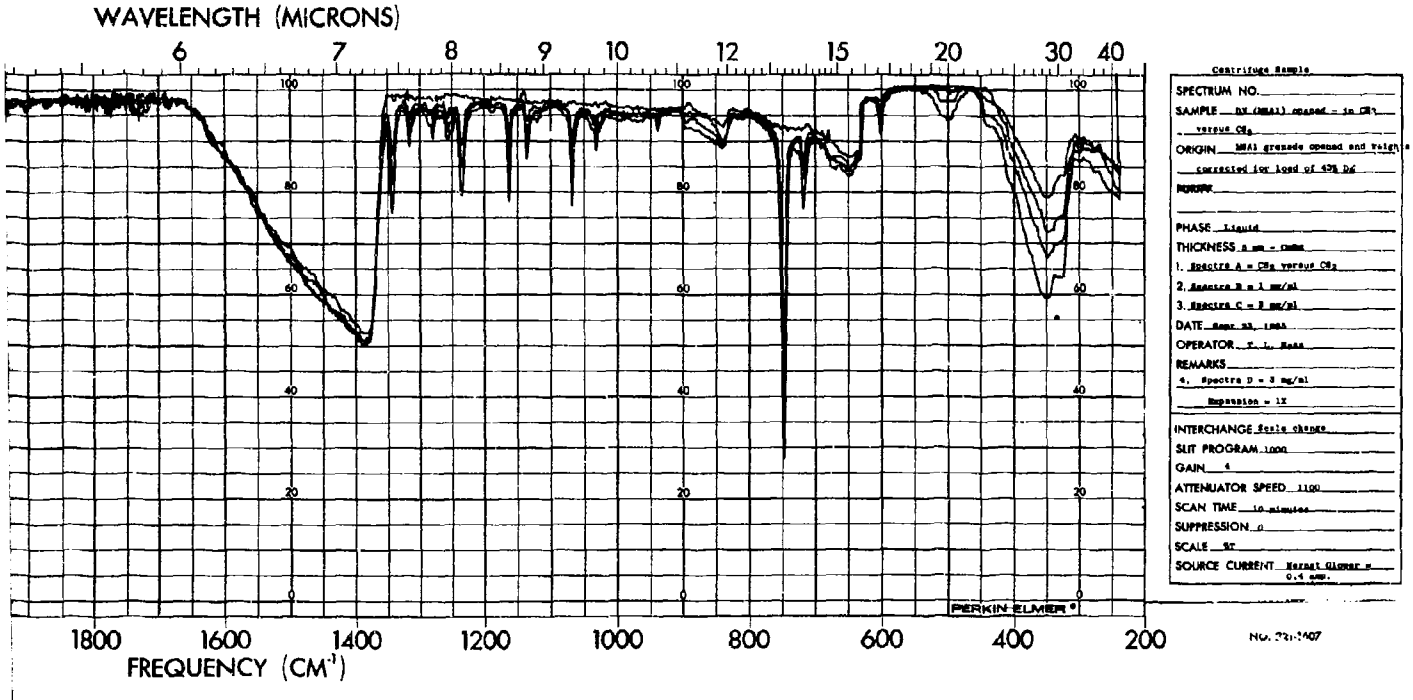


Figure A-5. Spectra for DM From an Opened M6A1 C



-5. Spectra for DM From an Opened M6A1 Grenade

2

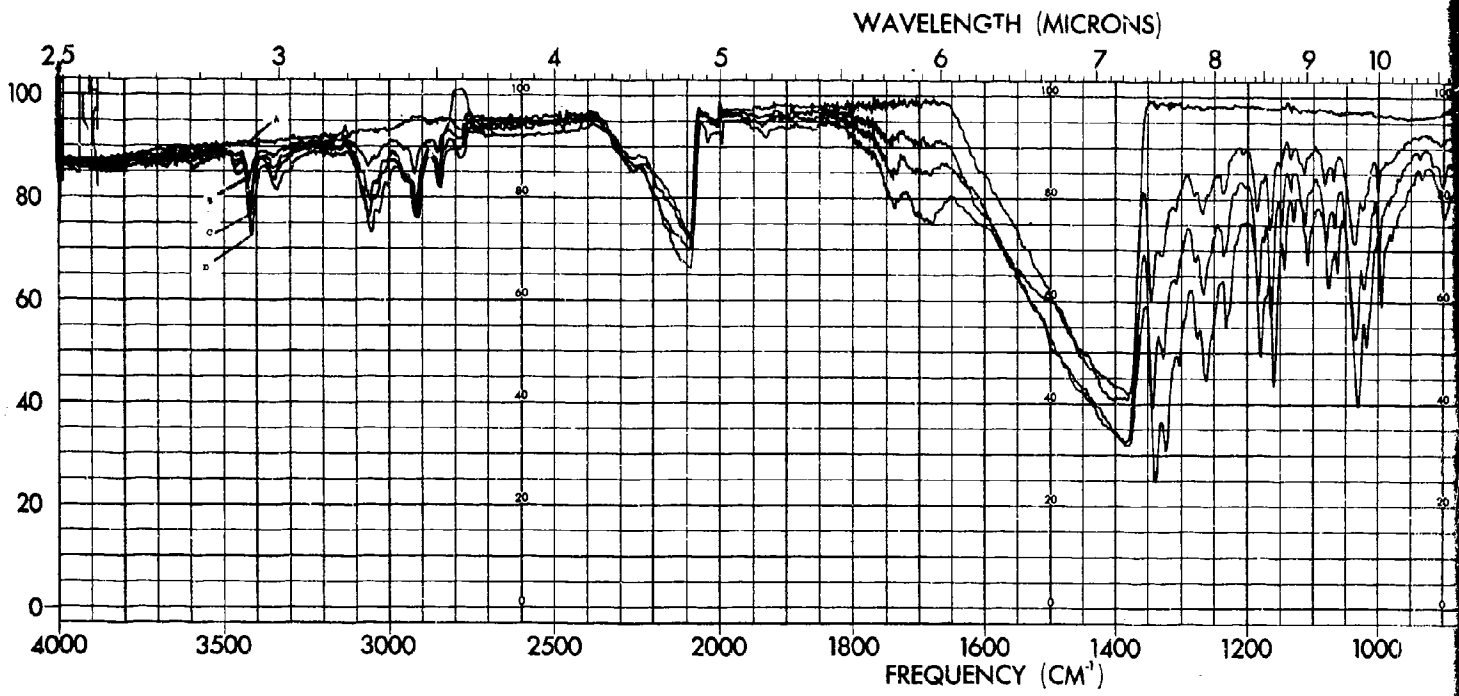
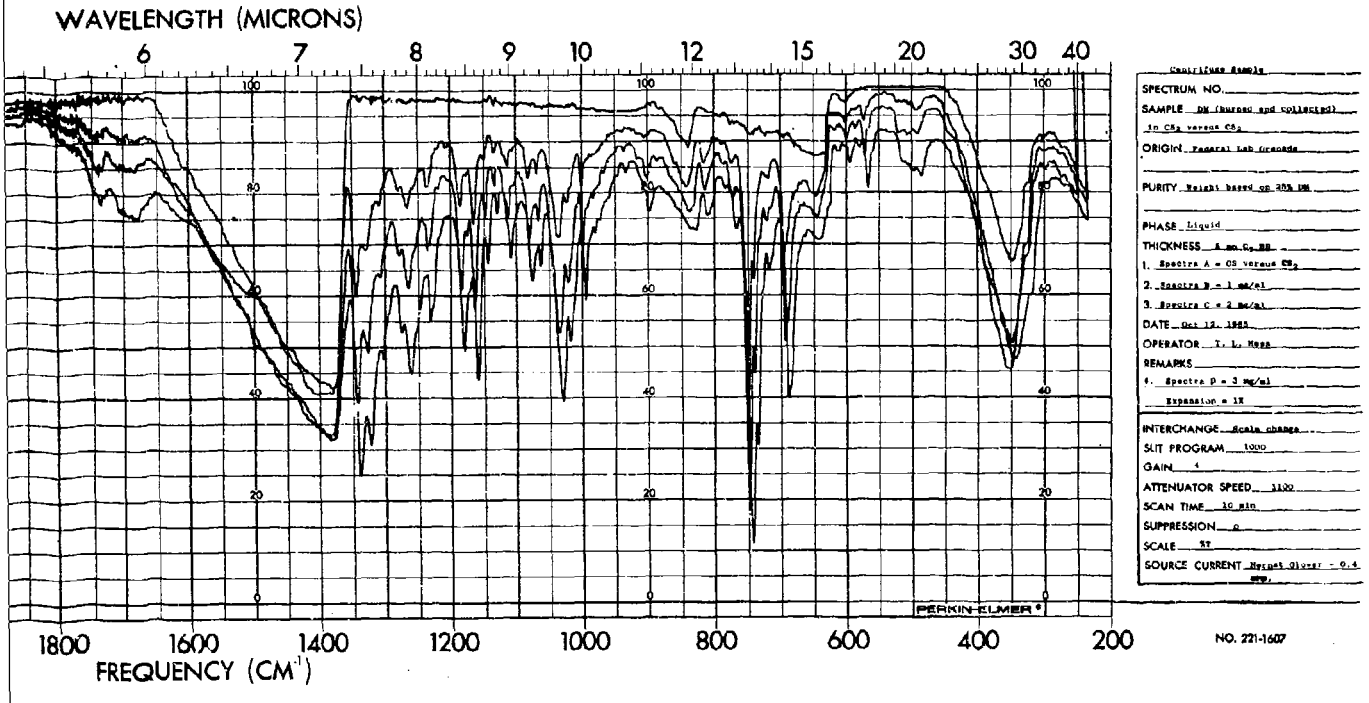


Figure A-6. Spectra for DM From a Burned No. 113 Gr



Spectra for DM From a Burned No. 113 Grenade

2

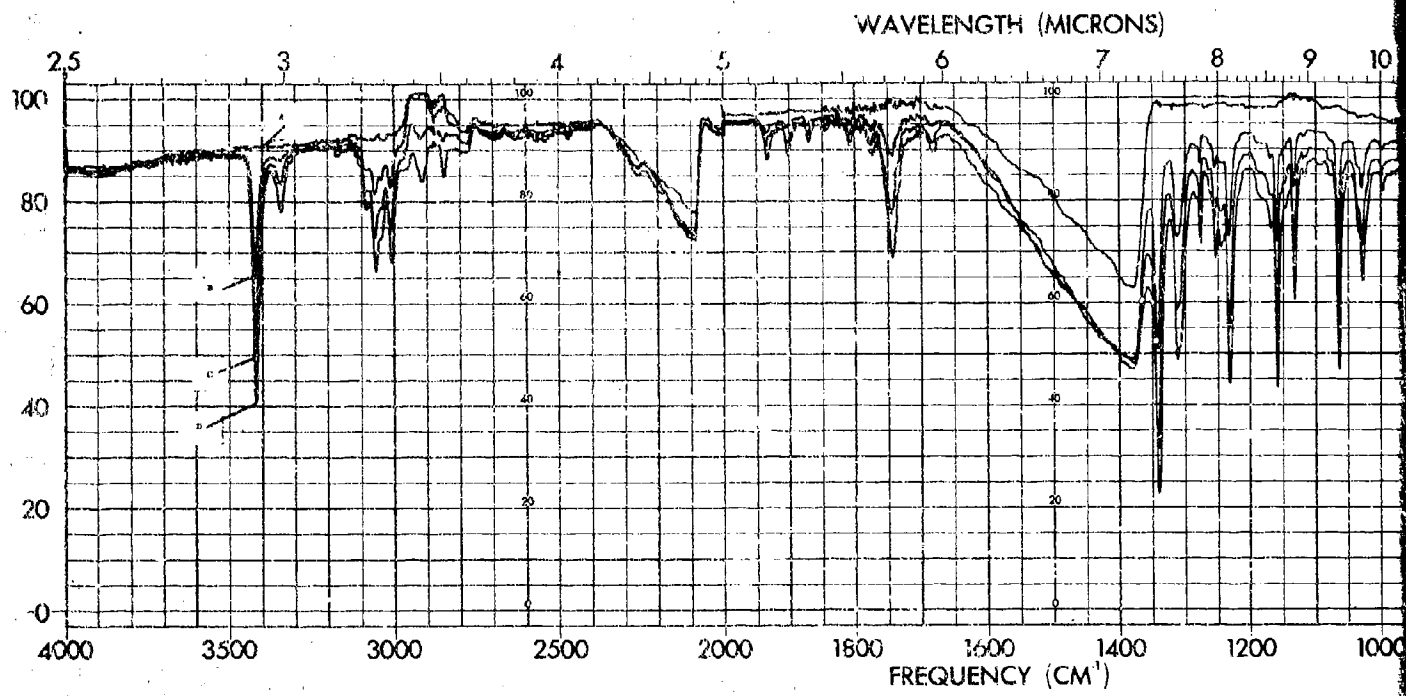
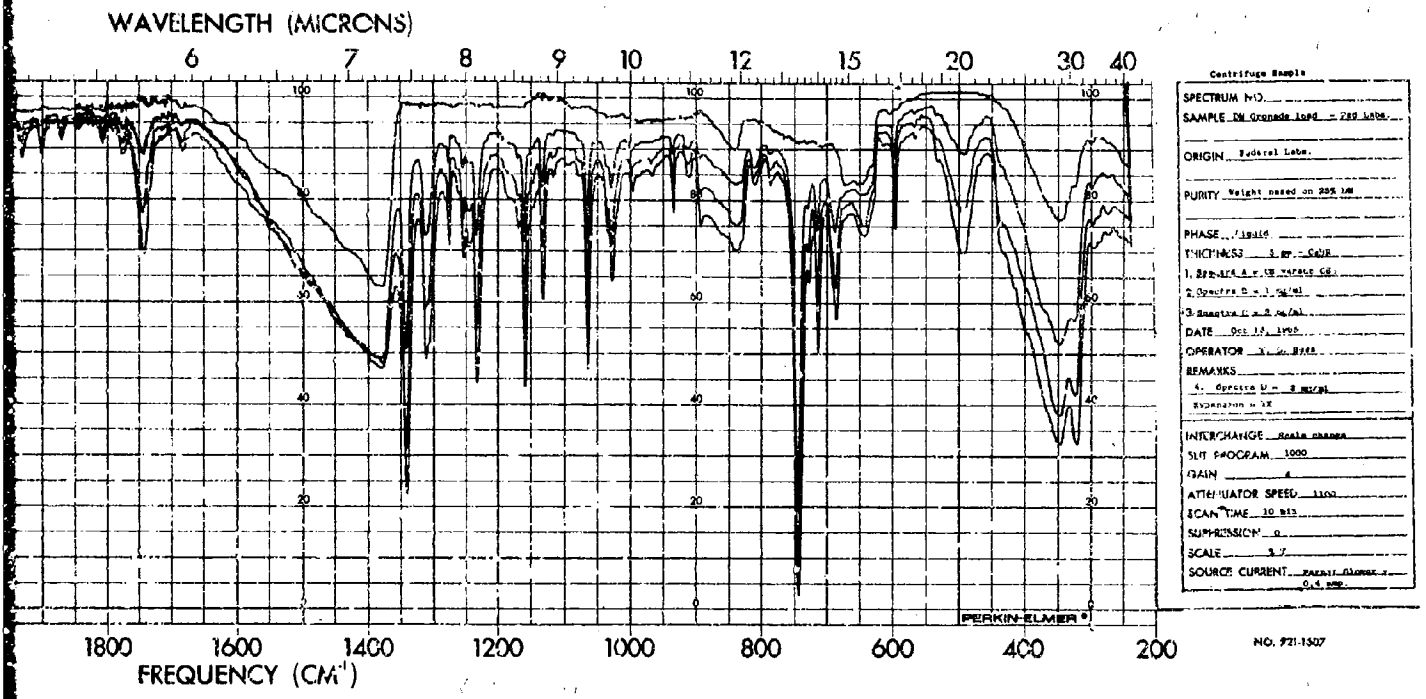


Figure A-7. Spectra for DM From an Opened No. 11



7. Spectra for DM From an Opened No. 113 Grenade

2

The UV absorbance spectrum for DM dissolved in absolute ethanol or in polyethylene glycol-200 (PEG-200) was the same. Absorbance maxima appear about 344, 308, and 276 mμ as shown in figure A-8.

The absorbance followed Beer's law at each peak. Solutions made to contain different concentrations of each of the samples mentioned produced absorbances proportional to those varying quantities of sample. This linear relationship persisted despite differences in strength of solutions or despite the presence of contaminants contained in the unburned grenades or in the smoke emanating from the burned grenade. This can be seen in tables A-II to A-IV.

The percentage of DM (by weight) in the various samples as determined by UV absorption and bioassay in rats is shown in table A-V.

The percentages of DM in various samples were studied by determination of lethality produced by iv injection of the material into rats. The lethality of these samples and the times to death in the rats tested are shown in table A-VI. The signs of intoxication and mortalities following the iv injection of the various DM types in rats are described in table A-VII.

6. Animal Observations.

The animal observations and pathological procedures followed by the Aerosol Branch between 1957 and 1965 are described by Punte and coworkers.[9] In some of these experiments, the animals were observed for signs during and after exposure. Histopathological examinations were performed on some of the animals sacrificed at the end of the observation periods. Times to death were recorded in some but not all experiments.

In the experiments performed during 1965, all animals were observed for signs during and for 30 days postexposure. Times to death, in hours, were recorded for all species. Representative numbers of survivors from each species were submitted for pathological examination at the end of the 30-day observation period. Also examined were representatives from each species at each Ct level that died during the observation period. Histopathological examinations were performed only on those animals exposed to DM disseminated from acetone solutions. Evaluation of these findings is in progress by personnel of the Veterinary Medicine Department, Medical Research Laboratory.

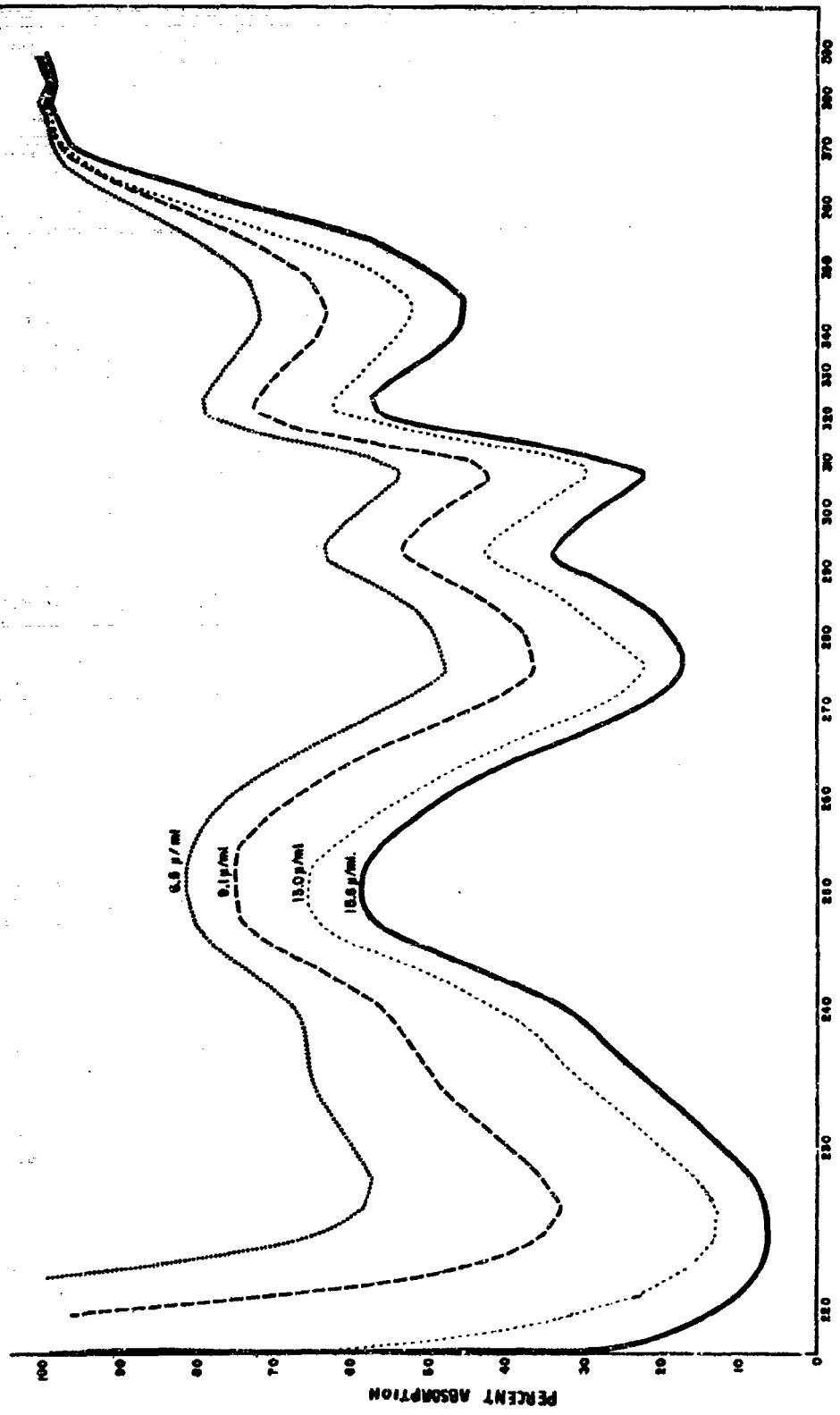


Figure A-8. UV Absorption Spectra of DM in Absolute Ethanol

Table A-II. UV Absorbance of a Series of Solutions of DM in Various Solvents at a Wavelength of Maximum Absorbance

Solvent	Maximum wavelength absorption	Concn standard solution	Maximum absorbance	
			Transmittance	Absorbance
	mμ	μ/ml	%	
	A. Pure DM (Unwashed)			
Absolute ethanol	344	6.5	72	0.143
	308		58	0.237
	277		48	0.319
	344	9.1	64	0.197
	307		43	0.372
	276		37	0.438
	344	13.0	53	0.276
	308		30	0.530
	275		23	0.648
	344	15.6	46	0.337
	308		23	0.638
	276		17	0.770
Polyethylene glycol	348	3.5	84	0.076
	309		73	0.140
	284		66	0.181
	348	7.0	73	0.140
	308		54	0.268
	283		45	0.347
	347	14.0	52	0.290
	308		28	0.560
	283		21	0.688
	348	21.0	36	0.450
	309		14	0.850
	284		8	1.126
	B. Pure DM (Washed)			
Sodium carbonate	283	5.5	53	0.276
	308		60	0.222
	348		76	0.119
	283	10.9	27	0.569
	308		33	0.482
	348		56	0.252
	283	16.4	15	0.837
	208		20	0.710
	348		43	0.368

Table A-III. UV Absorbance of Three Solutions of Burned and Unburned M6A1 Grenade Samples in PEG-200

Maximum wavelength absorption	Concn standard solution	Maximum absorbance	
		Transmittance	Absorbance
mμ	μ/ml	%	
A. Unburned M6A1 Grenade Sample			
283	7.7	76	0.119
308		79	0.102
348		88	0.056
283	15.4	56	0.252
308		61	0.215
348		77	0.114
283	30.9	29	0.545
308		37	0.438
348		59	0.229
B. First Burned M6A1 Grenade Sample			
283	4.3	75	0.128
308		77	0.116
348		89	0.051
283	8.6	56	0.256
308		59	0.229
348		79	0.105
283	17.2	31	0.509
308		35	0.456
348		61	0.215
C. Second Burned M6A1 Grenade Sample			
348	6.25	84	0.076
308		69	0.161
283		62	0.211
348	12.5	70	0.158
308		27	0.328
283		37	0.432
348	25.0	49	0.314
308		22	0.658
293		13	0.886

Table A-IV. UV Absorbance of Three Solutions of Unburned and Burned No. 113 Grenade Samples in PEG-200

Maximum wavelength absorption	Concn standard solution	Maximum absorbance	
		Transmittance	Absorbance
mμ	μ/ml	%	
A. Unburned No. 113 Grenade Sample			
283	8.1	78	0.108
308		78	0.108
348		91	0.041
283	25.0	41	0.387
308		53	0.276
348		78	0.111
283	32.3	33	0.482
308		36	0.444
348		67	0.174
B. Burned No. 113 Grenade Sample			
283	7.5	76	0.119
308		79	0.105
348		92	0.038
283	15.0	55	0.260
308		60	0.225
348		84	0.078
283	18.8	48	0.323
308		52	0.284
348		78	0.108

Table A-V. DM Content of Munitions

Sample	DM content* by weight		
	By formula	By absorbance 348 mμ	By bioassay compared with 95% DM as control
	%		
DM pure	100**	100	100
DM washed	102.4	106.3	121
Unburned M6A1 grenade	33.4	34.8	38
Burned M6A1 grenade (No. 1)	53.8	57.8	58
Burned M6A1 grenade (No. 2)	59.4	59.9	67
Unburned No. 113 grenade	24.7	23.5	297
Burned No. 113 grenade	25.4	25.3	38

* Mean values of three different solutions except for burned M6A1 grenade (No. 2).

** Original DM considered reference standard and assumed to be 100% DM.

Table A-VI. Iv Toxicity in Rats of Various Samples of DM

DM sample	Dose	Mortality fraction	Times to death	Statistical analysis				
				P	ED(P)	Lower limit	Upper limit	SE of slope
	mg/kg		days					
DM starting material	10.3	3/6	1, 4, 6	1	7.296	3.552	14.986	3.705
	11.8	2/6	1, 2	16	10.005	7.403	13.522	
	13.6	2/6	1(2)*	30	11.184	9.399	13.308	
	15.6	6/6	1(5), 6	50	12.664	11.060	14.501	
	18.0	6/6	1(5), 2	84	16.029	10.790	23.814	
				99	21.091	9.681	49.905	
Washed with solution of $NaHCO_3$ and distilled H_2O	7.4	0/6	–	1	7.140	2.792	18.258	4.553
	9.4	2/6	<1, 1	16	8.914	5.814	13.666	
	11.8	4/6	1(4)	30	9.640	7.435	14.499	
	14.8	6/6	<1, 1(5)	50	10.520	9.134	12.116	
				84	12.426	8.277	18.623	
				99	15.501	6.202	38.743	
Unburned M6A1 grenade sample	20.0	0/6	–	1	21.141	13.798	32.391	3.259
	23.7	1/6	1	16	27.418	22.212	23.843	
	28.2	0/6	–	30	30.051	25.840	34.950	
	33.5	3/6	<1(2), 1	50	33.291	29.399	37.698	
	39.9	5/6	<1(3), 1, 3	84	40.420	32.335	50.527	
	47.4	6/6	<1(6)	99	52.422	33.712	81.520	
Burned M6A1 grenade (No. 1)	15.6	0/6	–	1	17.464	9.990	30.530	7.347
	18.0	0/6	–	16	19.991	15.605	25.609	
	20.6	2/6	<1, 1	30	20.967	18.099	24.290	
	23.7	4/6	<1, 1(2), 2	50	22.113	20.294	24.095	
	27.4	6/6	<1(2), 1(4)	84	24.469	18.873	31.701	
	31.6	6/6	<1(2), 1(4)	99	27.999	15.821	49.553	
Burned M6A1 grenade (No. 2)	15.6	1/6	7	1	11.707	5.116	26.792	3.628
	18.0	3/6	1, 2, 3	16	16.480	10.356	23.139	
	20.6	3/6	1(3)	30	17.083	13.177	22.148	
	23.7	5/6	1(5)	50	19.069	16.665	21.821	
	27.4	6/6	1(5), 2	84	23.490	17.505	31.521	
	31.6	6/6	1(6)	99	31.060	15.210	63.429	
Unburned No. 113 grenade	5.1	6/6	1(6)	1	3.173	1.302	3.668	5.927
	4.4	2/6	1(2)	16	3.768	2.523	4.153	
	3.8	2/6	1(2)	30	4.003	3.120	4.432	
	3.3	0/6	–	50	4.283	3.749	5.025	
				84	4.868	4.404	7.691	
				99	5.780	4.962	14.971	
Burned No. 113 grenade	23.7	0/6	–	1	25.414	17.044	37.895	6.592
	28.2	0/6	–	16	29.876	24.521	36.402	
	33.5	4/6	<1(3), 1	30	31.632	27.561	36.305	
	39.8	5/6	<1(5)	50	33.713	30.431	37.349	
	47.4	6/6	<1(5), 1	84	38.042	31.889	45.812	
				99	44.722	30.396	65.801	

Note: Based on a 20-day observation.

* Number in parenthesis indicates number of mortalities at given time; otherwise, only one animal died at time indicated.

Table A-VII. Signs of Intoxication in Rats Following Iv Injection of Various Samples of DM

DM sample	Dose	No. of animals	Observable signs			
			Lacrimation	Irregular breathing	Nasal bloody exudate	Convulsions
	mg/kg					
Washed with solution of $NaHCO_3$ and diluted H_2O	7.4	6	3, 4, 6(3), 7			
	9.4	6	1, 2, 3, 4, 6(2)	9, 13, 15(3), 19	43	48
	11.8	6	3(2), 4, 6, 7, 9	10(2), 13, 15, 16		
	14.8	6	2, 4(2), 5, 6(2)	9, 10, 12, 13, 15, 16	14	16
Unburned M6A1	20.0	6	1, 2(3), 3(2)	4, 5(3), 6, 7		
	23.7	6	4, 5, 6(3), 7			
	28.2	6	1, 3, 5(2), 6, 9	9, 10, 13, 16, 17, 18		
	33.5	6	2, 3, 4(2), 5(2)	2, 3, 5(3), 6	10, 21	11, 24
	39.9	6	2(4), 3, 5	3(3), 4, 5, 8	17, 19, 48	19, 20, 112
	47.4	6	4(2), 5, 7, 9(2)	4, 5, 6, 8, 10, 11	6, 10, 13, 15, 20, 27	7, 13, 16, 23, 27, 31
Burned M6A1 grenade (No. 1)	15.6	6	5, 7(2), 11, 12, 27	27, 30		
	18.0	6	4, 5, 6, 7, 12(2)	12		
	20.6	6	2, 3, 4(3), 5	13, 21, 23, 26, 30, 31	93	104
	23.7	6	3(3), 6, 7(2)	6, 11, 16, 17, 18, 19	9	11
	27.4	6	1(2), 2(2), 3, 4	4, 5, 7, 10, 12, 13	11, 13	16, 17
	31.6	6	3, 4(2), 5, 6, 9	8, 10(3), 11, 12	11, 17	13, 30
Unburned No. 113 grenade	5.1	6	15, 24, 27, 48	29, 43, 69	55, 80, 113	148, 150, 247
	4.4	6				
	3.8	6				
	3.3	6				
Burned No. 113 grenade	23.7	6				
	28.2	6				
	33.5	6	4, 5, 6(2)	10(2), 17, 25	21, 30, 94	29, 34, 162
	39.8	6	2, 4(2), 8, 10			
	47.4	6	3(3), 6, 8, 9			

* Number in parenthesis indicates number of animals showing sign at particular time; otherwise, only one animal had sign at time indicated.

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APPENDIX B

PATHOLOGICAL FINDINGS

This section describes verbatim, the pathological examination of animals exposed to DM aerosols under Hazleton Laboratories Contract DA18-108-AMC-78(A) (reported in September 1963).

Dogs

The administration of pure DM did not produce any histologic changes in the organs of the animals receiving the lowest concentration (C = 107; Ct = 1610). In the next lowest concentration (C = 480; Ct = 14,000), the lung of one dog, at the end of one month, presented numerous foci of organizing granulation tissue in the walls of the small and medium bronchioles. Although this encroached on the lumen, it apparently did not completely occlude the passage because atelectasis was only slight and focal. The mucosa of the trachea of this dog was slightly infiltrated by leukocytes. In the lung of the other dog examined after one month, there was focal dilatation and collapse of alveolae and slight focal fibrous tissue thickening of the septa but the changes could not be definitely attributed to the compound. Alterations in the other organs of the dogs, of the 2 groups exposed to the lower concentration, presented no alterations attributable to the compound.

In the 3 higher concentrations, Trials No. 8, 3, and 1, the inhalation of the compound produced severe, acute ulcerative tracheitis and severe edema and congestion of the lungs, which resulted in the death of the animals within 24 hours in all 3 cases. As expected, congestion and small focal hemorrhages were described in several of the other organs. In addition, in the dogs exposed in Trial 3, there was acute degeneration of the gastric mucosa and focally in the small bowel mucosa. Passive congestion with centrilobular degeneration was moderate to severe in the livers of 3 dogs.

The brain of Dog No. 5946, exposed in Trial 2, presented an unusual lesion in the form of demyelination and focal gliosis in the blobus pallidus. The lesion was considered to be most likely secondary to acute anoxia that probably occurred during exposure. (The dog also exhibited ataxia during the period of recovery).

Monkeys

The administration of pure DM to monkeys at various concentrations resulted in pneumonitis starting with the animal exposed in Trial 2. In the lung of that monkey, there was a moderate degree of pneumonia with early organization compatible with the length of survival after the test.

After the administration of the compound at the next highest concentration, the monkey died within 24 hours, and the lung was severely edematous and slightly congested. There was ulcerative bronchiolitis and tracheitis, which was partly a result of aspiration of gastric contents as well as inhalation of the test compound. Monkey No. 14W, which survived 12 days after exposure in Trial 3, presented severe pneumonia and ulcerative tracheitis compatible with the length of survival after the test.

The administration of the compound produced no definite effect in the liver or kidney. Secondary changes in the spleen in the form of myelopoiesis were variable. A slight degree of hyperplasia of the lymphoid tissue was generally present. With regard to the latter finding, the extent of compound effect was questionable since the greatest degree of hyperplasia was found in the monkey which died within 24 hours after exposure.

No significant alterations were found in the brain, stomach, or small intestine.

The adrenal gland was generally not remarkable, aside from a low lipid level in the sona glomerulosa. In the highest level animal, there was a fairly large area of focal calcification in the inner portion of the cortex which was compatible with preexisting focal necrosis, probably occurring during the acute phase of the experiment.

The lymphoid tissue in the hilar lymph node was also hyperplastic and, in some instances, there was infiltration by granulocytes. The lymph node usually contained a fair amount of lung mite pigment.

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11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
Nondefense medical aspects of chemical agents	N/A

13. ABSTRACT

(U) This report summarizes the toxicological testing of diphenylaminochloroarsine (DM) in animals during the period from 1918 to 1965. Included are determinations of the toxicity of the compound disseminated by laboratory methods in early work and from military and commercially available thermal munitions in later work. The most probable human LCt50 estimates are derived from these experiments for the various methods of dissemination. All work described under the animal-testing section of the report pertains to either field or chamber total body exposures of eight species of test animals. Other portions of the toxicity studies deal with the pathological changes in exposed animals, times to death, and toxic responses. All available information on human exposure to DM, including accidental exposure of US and alien troops and Army personnel, is included.

14. KEYWORDS

DM	Dissemination	Chamber exposures
Animals	Munitions	M6A1 grenade
Toxicity	LCt50	Federal Laboratories No. 113
Toxicology	Human exposures	Spedeheat grenade
Summary report	Thermal munitions	
Diphenylaminochloroarsine	Field exposures	

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